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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12N 15/12, C07K 14/705, 14/715, A61K 38/17 — A1

(11) International Publication Number: WO 97/47741

(43) International Publication Date: 18 December 1997 (18.12.97)

(21) International Application Number: PCT/US96/10262

(22) International Filing Date: 12 June 1996 (12.06.96)

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(81) Designated States: AL, AM, AU, BB, BG, BR, CA, CN, CZ, EE, GE, HU, IL, IS, JP, KG, KP, KR, LK, LR, LT, LV, MD, MG, MK, MN, MX, NO, NZ, PL, RO, SG, SI, SK, TR, TT, UA, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: HR-1 RECEPTOR

(57) Abstract

Humain HR-1 RECEPTOR polypeptides and DNA (RNA) encoding such HR-1 RECEPTOR genes and a procedure for producing such polypeptides by recombinant techniques is disclosed. Also disclosed are methods for utilizing such HR-1 RECEPTORS for the treatment of decreased level of resistance to infection, asthma, various allergic and hematopoietic disorders or a susceptibility to the aforementioned abnormalities. Antagonists against such HR-1 RECEPTOR and their use as a therapeutic to treat decreased level of resistance to infection, asthma, various allergic and hematopoietic disorders or a susceptibility to the aforementioned abnormalities are aslo disclosed. Also disclosed are diagnostic assays for detecting diseases related to mutations in the nucleic acid sequences and altered concentrations of the polypeptides. Also disclosed are diagnostic assays for detecting mutations in the polynucleotides encoding the HR-1 RECEPTOR and for detecting altered levels of the polypeptide in a host.

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HR-1 RECEPTOR

FIELD OF THE INVENTION

This invention relates, in part, to newly identified polynucleotides and polypeptides; variants and derivatives of the polynucleotides and polypeptides; processes for making the polynucleotides and the polypeptides, and their variants and derivatives; agonists and antagonists of the polypeptides; and uses of the polynucleotides, polypeptides, variants, derivatives, agonists and antagonists. In particular, in these and in other regards, the invention relates to polynucleotides and polypeptides of human novel cytokine/peptide hormone receptors, hereinafter referred to as HR-1 RECEPTORS.

BACKGROUND OF THE INVENTION

The principle manner by which cells of the immune system communicate is through the elaboration of soluble factors. These growth and differentiation factors are essentially hormones made by the cells of the immune system. These factors are called cytokines. (As used herein cytokines include what are commonly called lymphokines). Cytokines act on virtually all cells of the hematopoietic system to regulate their growth and differention and on many non-hematopoietic cells as well, and play vital physiological vital role. Table I lists the molecules that are representative from this group.

TABLE 1

Cytokines and Their Receptors

The interleukin-4 family
IL-4
IL-5
GM-CSF
IL-3

Interleukin-6 related cytokines

IL-6

Granulocyte colony-stimulating factor

Oncostain M

Leukemia inhibitory factor

Ciliary neurotrophic factor

Other cytokines

EPO

IL-2

IL-7

IL-12

GH

Prolactin

Interferon-α

Interferon-β

Interferon-γ

Cytokines using immunoglobulin-like receptors

IL-1

Macrophage colony-stimulating factor

Platelet-derived growth factor

Stem cell factor, steel factor, or c-kit ligand

Cytokines using nerve growth factor-related receptors

Tumor necrosis factor-α

Lymphotoxin

Cytokines with uncharacterized receptors

IL-9

IL-10

IL-11

The list of cytokines grows constantly as new species are being discovered. A brief summary of what each cytokine's physiological role is given *in Immunology, A*

Synthesis, E. S. Golub and D. R. Green, 2nd Edition, Sinauer Assocaites, Inc., Sunderland, Massachusetts, pp 446-448 (1991). The physiological importance that various cytokines play can, in part, be seen from the function of the colony-stimulating factors (CSF's). CSF's were discovered because of their ability to stimulate the formation of colonies of granulocytes and macrophages in semisolid cultures of bone marrow cells. The CSF's are produced by multiple cell types, including fibroblasts, endothelial cells, stromal cells, and lymphocytes, that are widely distributed throughout the body. The levels of CSF product are normally low, but production can be rapidly elevated in response to emergencies such as the occurrence of an infection. Clinical trials on the GM-CSF and G-CSF have shown that they were able to elicit rises in blood and marrow granulocyte-macrophage populations without major toxicity. These results mean that GM-CSF and G-CSF or their analogs have potential utility in treating subnormal hematopoiesis, brought on either as a consequence of diseases such as acquired immunodeficiency syndrome (AIDS), aplastic anemia, congenital or cyclic neutropenia or as a consequence of cytotoxic therapy of cancer, lymphoma, or leukemia. Rapid regeneration of hematopoietic cells induced by CSF's or their analogs after cytotoxic therapy, as after bone marrow transplantation, for example can result in a shortening of the period of intensive nursing and hopsitalization. Because CSF's activate granulocytes and monocytes, it can also increase individuals' resistance to infections; such individuals include those with trauma or burns, or those scheduled for operations with a known risk of secondary infections. One response noted in individuals receiving CSF treatment is a rise up to 100-fold in the numbers of progenitor cells in the blood. These reach concentrations comparable with those in the bone marrow, raising the possiblity of using blood cells in place of or in addition to the marrow cells that are used for autologous transplantation. Another major application of CSF or their analogs stems from clinical trials which have shown not only white cells are regenerated by CSF but also platelets. This observation leads to a major practical importance because thrombocytopenia and the consequent need for platelets transfections remain major clinical problems in individuals receiving chemotraphy for cancer. Other medical applications for cytokines or their analogs are enormous, which include in areas such as a variety of

allergic disorders and asthma, since cytokines are directly or indirectly implicated in the pathogenesis of a wide variety of these disease.

Thus is a need for identification and characterization of new cytokines and their analogs which have important medical values in increasing level of resistance to infection in such individuals with burns or trauma, and preventing, ameliorating, treating, diagnosing, and/or determining predispostion to asthma, allergic disorders or disorders of hematopoiesis induced such as by AIDS, aplastic anemia, congenital or cyclic neutropenia or as a consequence of cytotoxic therapy of cancer, lymphoma, leukemia, and/or bone marrow transplantation. Clearly, there is clearly a need for factors which allow the identification of new cytokines which play important roles in the dysfunction and diseases related to allergies, asthma and various hematopoietic disorders caused such as by AIDS, aplastic anemia, congenital or cyclic neutropenia or as a consequence of cytotoxic therapy of cancer, lymphoma, leukemia, bone marrow transplantation, trauma and/or burns. (For general review of cytokines, see J.L. Boulay and W.E. Paul, *The Interleukin-4-related Lymphokines and Their Binding to Hematopoietin Receptors*, The Journal of Biological Chemistry, **267**, pp 20525-20528 (1992); and J.F. Bazan, *Structural Design and Molecular Evolution of a Cytokine Receptor Superfamily*, Proc. Natl. Acad. Sci., 87, pp 6934-6938 (1990)).

The ability of cytokines to influence the course of cell growth and differentiation uniquely depends on their recognition and binding to specific receptors; these cell surface molecules transduce the binding of messenger cytokines into cytoplasmic signals that trigger the developmental process within the cell. The sequences of hematopoietic cytokines do not appear to be related; in contrast, the family of cognate receptors reveals a striking resemblance of binding domains. The extracellular segments of the interleukin (IL) 2, 3, 4, 6, and 7, C-CSF and GM-CSF, TPO (thrombopoietin), leptin and erythropoietin (EPO) receptors share about 200 amino acid modules that show a distinctive conservation of four cysteine residues in the N-terminal half and a "WSxWS" box (one-letter amino acid code; x is a nonconserved residue) near the C-terminal end. Similar motif mark homologous domains in growth hormone (GRH) and prolactin (PRL) receptors.

The polypeptide of the present invention has amino acid sequence homology to known GM-CSF receptor, prolactin receptor, IL-3 receptor. In addition, the cytokine receptor type 1 motif is conserved in the polypeptide of the present invention, indicating the present polypeptide is a novel cytokine/peptide hormone receptor (HR-1 RECEPTOR). In particular the HR-1 RECEPTOR of the present invention 27 % amino acid sequence identity and 52 % similarity with the known human IL-5 receptor.

Interleukin 5 (IL-5) is a hematopoietic growth factor protein which plays a key role in the proliferation and activation of eosinophils. Increased levels and inappropriate accumulation of eosinophils in sites such as the lung have implicated these cells in inflammatory diseases such as asthma. Since IL-5 acts on eosinophils and their progenitor cells through a cell surface receptor, the lineage specific effects of hIL5 on eosinophils have generated intense interest to characterize the hIL-5 receptor interaction and to identify antagonists for this process. See, D. Bennett et al., *Journal of Molecular Recognition* , Vol. 8, pp 52-58 (1995) and references cited therein.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide polypeptides, *inter alia*, that have been identified as novel HR-1 RECEPTOR with amino acid sequences shown in Figure 1 (SEQ ID NO:[2]).

It is a further object of the invention, moreover, to provide polynucleotides that encode HR-1 RECEPTOR, particularly polynucleotides that encode the polypeptide herein designated HR-1 RECEPTOR.

In a particularly preferred embodiment of this aspect of the invention the polynucleotide comprises the region encoding human HR-1 RECEPTOR in the sequence set out in Figure 1 (SEQ ID NO: [2]).

In accordance with this aspect of the present invention there is provided an isolated nucleic acid molecule encoding a mature polypeptide expressed by the human cDNA contained in vector pBluescriptSK+, plamid ATG-531, in E. coli JM101 strain ATCC Deposit No. 98069.

In accordance with this aspect of the invention there are provided isolated nucleic acid molecules encoding human HR-1 RECEPTOR, including mRNAs, cDNAs, genomic DNAs and, in further embodiments of this aspect of the invention, biologically, diagnostically, clinically or therapeutically useful variants, analogs or derivatives thereof, or fragments thereof, including fragments of the variants, analogs and derivatives.

Among the particularly preferred embodiments of this aspect of the invention are naturally occurring allelic variants of human HR-1 RECEPTOR.

It also is an object of the invention to provide HR-1 RECEPTOR polypeptides, particularly human HR-1 RECEPTOR polypeptides, that may be employed for clinical purposes, for example, to increase level of resistance to infection in individuals such as with trauma and/or burns, and to prevent, ameliorate, treat, diagnose, and/or determine predisposition to asthma, allergic disorders or disorders of hematopoiesis induced such as by AIDS, aplastic anemia, congenital or cyclic neutropenia or as a consequence of cytotoxic therapy of cancer, lymphoma, leukemia, and/or bone marrow transplantation.

In accordance with this aspect of the invention there are provided novel polypeptides of human origin referred to herein as HR-1 RECEPTOR as well as biologically, diagnostically or therapeutically useful fragments, variants and derivatives thereof, variants and derivatives of the fragments, and analogs of the foregoing.

Among the particularly preferred embodiments of this aspect of the invention are variants of human HR-1 RECEPTOR encoded by naturally occurring alleles of the human HR-1 RECEPTOR gene.

In accordance with another aspect of the present invention there are provided methods of screening for compounds (including ligands or other proteins) which bind to and activate or inhibit activation of the receptor polypeptides of the present invention and for receptor ligands.

It is another object of the invention to provide a process for producing the aforementioned polypeptides, polypeptide fragments, variants and derivatives, fragments of the variants and derivatives, and analogs of the foregoing.

In a preferred embodiment of this aspect of the invention there are provided methods for producing the aforementioned HR-1 RECEPTOR polypeptides comprising culturing host cells having expressibly incorporated therein an exogenously-derived human HR-1 RECEPTOR-encoding polynucleotide under conditions for expression of human HR-1 RECEPTOR in the host and then recovering the expressed polypeptide.

In accordance with another object the invention there are provided products, compositions, processes and methods that utilize the aforementioned polypeptides and polynucleotides for research, biological, clinical and therapeutic purposes, *inter alia*, to increase level resistance to infection in individuals such as with trauma and/or burns, and to prevent, ameliorate, treat, diagnose, and/or determine predisposition to asthma, allergic disorders or disorders of hematopoiesis induced such as by AIDS, aplastic anemia, congenital or cyclic neutropenia or as a consequence of cytotoxic therapy of cancer, lymphoma, leukemia, and/or bone marrow transplantation.

In accordance with certain preferred embodiments of this aspect of the invention, there are provided products, compositions and methods, inter alia, for, among other things: assessing HR-1 RECEPTOR expression in cells by determining HR-1 RECEPTOR polypeptides or HR-1 RECEPTOR-encoding mRNA; to increase level of resistance to infection in individuals such as with trauma and/or burns, and to prevent, ameliorate, treat, diagnose, and/or determine predisposition to asthma, allergic disorders or disorders of hematopoiesis induced such as by AIDS, aplastic anemia, congenital or cyclic neutropenia or as a consequence of cytotoxic therapy of cancer, lymphoma, leukemia, and/or bone marrow transplantation *in vitro, ex vivo* or *in vivo* by exposing cells to HR-1 RECEPTOR polypeptides or polynucleotides as disclosed herein; assaying genetic variation and aberrations, such as defects, in HR-1 RECEPTOR genes; and administering a HR-1 RECEPTOR polypeptide or polynucleotide to an organism to augment HR-1 RECEPTOR function or remediate HR-1 RECEPTOR dysfunction.

In accordance with still another embodiment of the present invention there is provided a process of using such activating compounds to stimulate the receptor

polypeptide of the present invention for the treatment of conditions related to the under-expression of the HR-1 RECEPTOR.

In accordance with another aspect of the present invention there is provided a process of using such inhibiting compounds for treating conditions associated with over-expression of the HR-1 RECEPTOR.

In accordance with yet another aspect of the present invention there is provided non-naturally occurring synthetic, isolated and/or recombinant HR-1 RECEPTOR polypeptides which are fragments, consensus fragments and/or sequences having conservative amino acid substitutions such that the receptor may bind HR-1 RECEPTOR ligands, or which may also modulate, quantitatively or qualitatively, HR-1 RECEPTOR ligand binding.

In accordance with still another aspect of the present invention there are provided synthetic or recombinant HR-1 RECEPTOR polypeptides, conservative substitution and derivatives thereof, antibodies, anti-idiotype antibodies, compositions and methods that can be useful as potential modulators of HR-1 RECEPTOR function, by binding to ligands or modulating ligand binding, due to their expected biological properties, which may be used in diagnostic, therapeutic and/or research applications.

It is still another object of the present invention to provide synthetic, isolated or recombinant polypeptides which are designed to inhibit or mimic various HR-1 RECEPTOR or fragments thereof, as receptor types and subtypes.

In accordance with certain preferred embodiments of this and other aspects of the invention there are provided probes that hybridize to human HR-1 RECEPTOR sequences.

In certain additional preferred embodiments of this aspect of the invention there are provided antibodies against HR-1 RECEPTOR polypeptides. In certain particularly preferred embodiments in this regard, the antibodies are highly selective for human HR-1 RECEPTOR.

In accordance with another aspect of the present invention, there are provided HR-1 RECEPTOR agonists. Among preferred agonists are molecules that mimic HR-1 RECEPTOR, that bind to HR-1 RECEPTOR-binding molecules or receptor molecules, and that elicit or augment HR-1 RECEPTOR-induced responses. Also

among preferred agonists are molecules that interact with HR-1 RECEPTOR or HR-1 RECEPTOR polypeptides, or with other modulators of HR-1 RECEPTOR activities, and thereby potentiate or augment an effect of HR-1 RECEPTOR or more than one effect of HR-1 RECEPTOR.

In accordance with yet another aspect of the present invention, there are provided HR-1 RECEPTOR antagonists. Among preferred antagonists are those which mimic HR-1 RECEPTOR so as to bind to HR-1 RECEPTOR receptor or binding molecules but not elicit a HR-1 RECEPTOR-induced response or more than one HR-1 RECEPTOR-induced response. Also among preferred antagonists are molecules that bind to or interact with HR-1 RECEPTOR so as to inhibit an effect of HR-1 RECEPTOR or more than one effect of HR-1 RECEPTOR or which prevent expression of HR-1 RECEPTOR.

In a further aspect of the invention there are provided compositions comprising a HR-1 RECEPTOR polynucleotide or a HR-1 RECEPTOR polypeptide for administration to cells *in vitro*, to cells *ex vivo* and to cells *in vivo*, or to a multicellular organism. In certain particularly preferred embodiments of this aspect of the invention, the compositions comprise a HR-1 RECEPTOR polynucleotide for expression of a HR-1 RECEPTOR polypeptide in a host organism for treatment of disease. Particularly preferred in this regard is expression in a human patient for treatment of a dysfunction associated with aberrant endogenous activity of HR-1 RECEPTOR and endogenous activity of its ligands.

Other objects, features, advantages and aspects of the present invention will become apparent to those of skill in the art from the following description. It should be understood, however, that the following description and the specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only. Various changes and modifications within the spirit and scope of the disclosed invention will become readily apparent to those skilled in the art from reading the following description and from reading the other parts of the present disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the nucleotide and deduced amino acid sequence of human HR-1 RECEPTOR. The underlined amino acid sequence indicates signal sequence.

Figure 2 is an illustration of the amino acid homology between the polypeptide of the present invention (top line) and human IL-5 receptor.

GLOSSARY

The following illustrative explanations are provided to facilitate understanding of certain terms used frequently herein, particularly in the examples. The explanations are provided as a convenience and are not limitative of the invention.

DIGESTION of DNA refers to catalytic cleavage of the DNA with a restriction enzyme that acts only at certain sequences in the DNA. The various restriction enzymes referred to herein are commercially available and their reaction conditions, cofactors and other requirements for use are known and routine to the skilled artisan.

For analytical purposes, typically, 1 µg of plasmid or DNA fragment is digested with about 2 units of enzyme in about 20 µl of reaction buffer. For the purpose of isolating DNA fragments for plasmid construction, typically 5 to 50 µg of DNA are digested with 20 to 250 units of enzyme in proportionately larger volumes.

Appropriate buffers and substrate amounts for particular restriction enzymes are described in standard laboratory manuals, such as those referenced below, and they are specified by commercial suppliers.

Incubation times of about 1 hour at 37°C are ordinarily used, but conditions may vary in accordance with standard procedures, the supplier's instructions and the particulars of the reaction. After digestion, reactions may be analyzed, and fragments may be purified by electrophoresis through an agarose or polyacrylamide gel, using well known methods that are routine for those skilled in the art.

GENETIC ELEMENT generally means a polynucleotide comprising a region that encodes a polypeptide or a region that regulates transcription or translation or other processes important to expression of the polypeptide in a host cell, or a

polynucleotide comprising both a region that encodes a polypeptide and a region operably linked thereto that regulates expression.

Genetic elements may be comprised within a vector that replicates as an episomal element; that is, as a molecule physically independent of the host cell genome. They may be comprised within mini-chromosomes, such as those that arise during amplification of transfected DNA by methotrexate selection in eukaryotic cells. Genetic elements also may be comprised within a host cell genome; not in their natural state but, rather, following manipulation such as isolation, cloning and introduction into a host cell in the form of purified DNA or in a vector, among others.

ISOLATED means altered "by the hand of man" from its natural state; i.e., that, if it occurs in nature, it has been changed or removed from its original environment, or both.

For example, a naturally occurring polynucleotide or a polypeptide naturally present in a living animal in its natural state is not "isolated," but the same polynucleotide or polypeptide separated from the coexisting materials of its natural state is "isolated", as the term is employed herein. For example, with respect to polynucleotides, the term isolated means that it is separated from the chromosome and cell in which it naturally occurs.

As part of or following isolation, such polynucleotides can be joined to other polynucleotides, such as DNAs, for mutagenesis, to form fusion proteins, and for propagation or expression in a host, for instance. The isolated polynucleotides, alone or joined to other polynucleotides such as vectors, can be introduced into host cells, in culture or in whole organisms. Introduced into host cells in culture or in whole organisms, such DNAs still would be isolated, as the term is used herein, because they would not be in their naturally occurring form or environment. Similarly, the polynucleotides and polypeptides may occur in a composition, such as a media formulations, solutions for introduction of polynucleotides or polypeptides, for example, into cells, compositions or solutions for chemical or enzymatic reactions, for instance, which are not naturally occurring compositions, and, therein remain isolated polynucleotides or polypeptides within the meaning of that term as it is employed herein.

LIGATION refers to the process of forming phosphodiester bonds between two or more polynucleotides, which most often are double stranded DNAs. Techniques for ligation are well known to the art and protocols for ligation are described in standard laboratory manuals and references, such as, for instance, Sambrook et al., MOLECULAR CLONING, A LABORATORY MANUAL, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989) and Maniatis et al., pg. 146, as cited below.

OLIGONUCLEOTIDE(S) refers to relatively short polynucleotides. Often the term refers to single-stranded deoxyribonucleotides, but it can refer as well to single-or double-stranded ribonucleotides, RNA:DNA hybrids and double-stranded DNAs, among others.

Oligonucleotides, such as single-stranded DNA probe oligonucleotides, often are synthesized by chemical methods, such as those implemented on automated oligonucleotide synthesizers. However, oligonucleotides can be made by a variety of other methods, including in vitro recombinant DNA-mediated techniques and by expression of DNAs in cells and organisms.

Initially, chemically synthesized DNAs typically are obtained without a 5' phosphate. The 5' ends of such oligonucleotides are not substrates for phosphodiester bond formation by ligation reactions that employ DNA ligases typically used to form recombinant DNA molecules. Where ligation of such oligonucleotides is desired, a phosphate can be added by standard techniques, such as those that employ a kinase and ATP.

The 3' end of a chemically synthesized oligonucleotide generally has a free hydroxyl group and, in the presence of a ligase, such as T4 DNA ligase, readily will form a phosphodiester bond with a 5' phosphate of another polynucleotide, such as another oligonucleotide. As is well known, this reaction can be prevented selectively, where desired, by removing the 5' phosphates of the other polynucleotide(s) prior to ligation.

PLASMIDS generally are designated herein by a lower case p preceded and/or followed by capital letters and/or numbers, in accordance with standard naming conventions that are familiar to those of skill in the art.

Starting plasmids disclosed herein are either commercially available, publicly available on an unrestricted basis, or can be constructed from available plasmids by routine application of well known, published procedures. Many plasmids and other cloning and expression vectors that can be used in accordance with the present invention are well known and readily available to those of skill in the art. Moreover, those of skill readily may construct any number of other plasmids suitable for use in the invention. The properties, construction and use of such plasmids, as well as other vectors, in the present invention will be readily apparent to those of skill from the present disclosure.

POLYNUCLEOTIDE(S) generally refers to any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. Thus, for instance, polynucleotides as used herein refers to, among others, single-and double-stranded DNA, DNA that is a mixture of single-and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions.

In addition, polynucleotide as used herein refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The strands in such regions may be from the same molecule or from different molecules. The regions may include all of one or more of the molecules, but more typically involve only a region of some of the molecules. One of the molecules of a triple-helical region often is an oligonucleotide.

As used herein, the term polynucleotide includes DNAs or RNAs as described above that contain one or more modified bases. Thus, DNAs or RNAs with backbones modified for stability or for other reasons are "polynucleotides" as that term is intended herein. Moreover, DNAs or RNAs comprising unusual bases, such as inosine, or modified bases, such as tritylated bases, to name just two examples, are polynucleotides as the term is used herein.

It will be appreciated that a great variety of modifications have been made to DNA and RNA that serve many useful purposes known to those of skill in the art. The term polynucleotide as it is employed herein embraces such chemically, enzymatically or metabolically modified forms of polynucleotides, as well as the chemical forms of

DNA and RNA characteristic of viruses and cells, including simple and complex cells, inter alia.

POLYPEPTIDES, as used herein, includes all polypeptides as described below. The basic structure of polypeptides is well known and has been described in innumerable textbooks and other publications in the art. In this context, the term is used herein to refer to any peptide or protein comprising two or more amino acids joined to each other in a linear chain by peptide bonds. As used herein, the term refers to both short chains, which also commonly are referred to in the art as peptides, oligopeptides and oligomers, for example, and to longer chains, which generally are referred to in the art as proteins, of which there are many types.

It will be appreciated that polypeptides often contain amino acids other than the 20 amino acids commonly referred to as the 20 naturally occurring amino acids, and that many amino acids, including the terminal amino acids, may be modified in a given polypeptide, either by natural processes, such as processing and other post-translational modifications, but also by chemical modification techniques which are well known to the art. Even the common modifications that occur naturally in polypeptides are too numerous to list exhaustively here, but they are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature, and they are well known to those of skill in the art.

Among the known modifications which may be present in polypeptides of the present are, to name an illustrative few, acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphotidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cystine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination.

Such modifications are well known to those of skill and have been described in great detail in the scientific literature. Several particularly common modifications, glycosylation, lipid attachment, sulfation, gamma-carboxylation of glutamic acid residues, hydroxylation and ADP-ribosylation, for instance, are described in most basic texts, such as, for instance PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York (1993). Many detailed reviews are available on this subject, such as, for example, those provided by Wold, F., Posttranslational Protein Modifications: Perspectives and Prospects, pgs. 1-12 in POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York (1983); Seifter et al., Analysis for protein modifications and nonprotein cofactors, Meth. Enzymol. 182: 626-646 (1990) and Rattan et al., Protein Synthesis: Posttranslational Modifications and Aging, Ann. N.Y. Acad. Sci. 663: 48-62 (1992).

It will be appreciated, as is well known and as noted above, that polypeptides are not always entirely linear. For instance, polypeptides may be branched as a result of ubiquitination, and they may be circular, with or without branching, generally as a result of posttranslation events, including natural processing event and events brought about by human manipulation which do not occur naturally. Circular, branched and branched circular polypeptides may be synthesized by non-translation natural process and by entirely synthetic methods, as well.

Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. In fact, blockage of the amino or carboxyl group in a polypeptide, or both, by a covalent modification, is common in naturally occurring and synthetic polypeptides and such modifications may be present in polypeptides of the present invention, as well. For instance, the amino terminal residue of polypeptides made in E. coli, prior to proteolytic processing, almost invariably will be N-formylmethionine.

The modifications that occur in a polypeptide often will be a function of how it is made. For polypeptides made by expressing a cloned gene in a host, for instance, the nature and extent of the modifications in large part will be determined by the host cell posttranslational modification capacity and the modification signals present in the

polypeptide amino acid sequence. For instance, as is well known, glycosylation often does not occur in bacterial hosts such as E. coli. Accordingly, when glycosylation is desired, a polypeptide should be expressed in a glycosylating host, generally a eukaryotic cell. Insect cell often carry out the same posttranslational glycosylations as mammalian cells and, for this reason, insect cell expression systems have been developed to express efficiently mammalian proteins having native patterns of glycosylation, inter alia. Similar considerations apply to other modifications.

It will be appreciated that the same type of modification may be present in the same or varying degree at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications.

In general, as used herein, the term polypeptide encompasses all such modifications, particularly those that are present in polypeptides synthesized by expressing a polynucleotide in a host cell.

VARIANT(S) of polynucleotides or polypeptides, as the term is used herein, are polynucleotides or polypeptides that differ from a reference polynucleotide or polypeptide, respectively. Variants in this sense are described below and elsewhere in the present disclosure in greater detail.

(1) A polynucleotide that differs in nucleotide sequence from another, reference polynucleotide. Generally, differences are limited so that the nucleotide sequences of the reference and the variant are closely similar overall and, in many regions, identical.

As noted below, changes in the nucleotide sequence of the variant may be silent. That is, they may not alter the amino acids encoded by the polynucleotide. Where alterations are limited to silent changes of this type a variant will encode a polypeptide with the same amino acid sequence as the reference. Also as noted below, changes in the nucleotide sequence of the variant may alter the amino acid sequence of a polypeptide encoded by the reference polynucleotide. Such nucleotide changes may result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference sequence, as discussed below.

(2) A polypeptide that differs in amino acid sequence from another, reference polypeptide. Generally, differences are limited so that the sequences of the reference and the variant are closely similar overall and, in many region, identical.

A variant and reference polypeptide may differ in amino acid sequence by one or more substitutions, additions, deletions, fusions and truncations, which may be present in any combination.

FUSION PROTEINS: EP-A-O 464 533 (Canadian counterpart 2045869) discloses fusion proteins comprising various portions of constant region of immunoglobin molecules together with another human protein or part thereof. In many cases, the Fc part in fusion protein is thoroghly advantageous for use in therapy and diagnosis and thus results, for example, in improved pharmacokinetic properties (EP-A 0232 262). On the other hand, for some uses it would be desirable to be able to delete the Fc part after the fusion protein has been expressed, detected and purified in the advantageous manner described. This is the case when Fc portion proves to be a hindrance to use in therapy and diagnosis, for example when the fusion protein is to be used as antigen for immunizations. In drug discovery, for example, human proteins, such as, shIL5-α has been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. See, D. Bennett et al., *Journal of Molecular Recognition*, Vol. 8 52-58 (1995) and K. Johanson et al., *The Journal of Biological Chemistry*, Vol. 270, No. 16, pp 9459-9471 (1995).

Thus, this invention also relates to genetically engineered soluble fusion proteins comprised from HR-1 RECEPTOR, or a portion thereof, and of various portions of the constant regions of heavy or light chains of immunoglobulins of various subclass (IgG, IgM, IgA, IgE). Preferred as immunoglobulin is the constant part of the heavy chain of human IgG, particularly IgG1, where fusion takes place at the hinge region. In a particular embodiment, the Fc part can be removed in a simple way by a cleavage sequence which is also incorporated and can be cleaved with factor Xa. Furthermore, this invention relates to processes for the preparation of these fusion by genetic engineering, and to the use thereof for diagnosis and therapy.

RECEPTOR MOLECULE, as used herein, refers to molecules of the present invention, including but not limited to HR-1 RECEPTOR polyptides, as well as

molecules which bind or interact specifically with HR-1 RECEPTOR (ligands) polypeptides of the present invention, including not only classic receptors, which are preferred, but also other molecules that specifically bind to or interact with polypeptides of the invention (which also may be referred to as "binding molecules" and "interaction molecules," respectively and as "HR-1 RECEPTOR binding molecules" and "HR-1 RECEPTOR interaction molecules." Binding between polypeptides of the invention and such molecules, including receptor or binding or interaction molecules may be exclusive to polypeptides of the invention, which is very highly preferred, or it may be highly specific for polypeptides of the invention, which is highly preferred, or it may be highly specific to a group of proteins that includes polypeptides of the invention, which is preferred, or it may be specific to several groups of proteins at least one of which includes polypeptides of the invention.

Receptor molecules also may be non-naturally occuring, such as antibodies and antibody-derived reagents that bind specifically to polypetides of the invention.

DESCRIPTION OF THE INVENTION

The present invention relates to novel HR-1 RECEPTOR polypeptides and polynucleotides, among other things, as described in greater detail below. In particular, the invention relates to polypeptides and polynucleotides of a novel human HR-1 RECEPTOR, which is related by amino acid sequence homology to human IL-5 receptor polypeptide. The invention relates especially to HR-1 RECEPTOR having the nucleotide and amino acid sequence set out in Figure 1 (SEQ ID NO: 2), and to the HR-1 RECEPTOR nucleotide and amino acid sequences of the human cDNA in ATCC Deposit No. 98069, which is herein referred to as "the deposited clone" or as the "cDNA of the deposited clone." It will be appreciated that the nucleotide and amino acid sequence set out in Figure 1 (SEQ ID NO: 2) were obtained by sequencing the cDNA of the deposited clone. Hence, the sequence of the deposited clone is controlling as to any discrepancies between the two and any reference to the sequences of Figure 1 include reference to the sequence of the human cDNA of the deposited clone.

Polynucleotides

In accordance with one aspect of the present invention, there are provided isolated polynucleotides which encode the HR-1 RECEPTOR polypeptide having the deduced amino acid sequence of Figure 1 (SEQ ID NO: 2).

Using the information provided herein, such as the polynucleotide sequence set out in Figure 1 (SEQ ID NO: 1), a polynucleotide of the present invention encoding human HR-1 RECEPTOR polypeptide may be obtained using standard cloning and screening procedures, such as those for cloning cDNAs using mRNA from cells from microvascular endothelial tissue as starting material. Illustrative of the invention, the polynucleotide set out in Figure 1 (SEQ ID NO: 1) was discovered in a cDNA library derived from cells of human testes tissue.

Human HR-1 RECEPTOR of the invention is structurally related to other proteins of the cytokine and peptide hormone receptors, as shown by the results of sequencing the cDNA encoding human HR-1 RECEPTOR in the deposited clone. The cDNA sequence thus obtained is set out in Figure 1. It contains an open reading frame encoding a protein of about 380 amino acid residues with a deduced molecular weight of about 44.176 kDa. The protein exhibits greatest homology to human IL-5 receptor protein among known proteins. HR-1 RECEPTOR of Figure 1 has about 27% identity and about 52 % similarity with the amino acid sequence of IL-5 receptor protein.

Human HR-1 RECEPTOR of the present invention contain 21 amino acids signal sequence on the N-terminus. Thus another aspect of the invention relates to HR-1 RECEPTOR without the signal sequence with the amino acid residues 22 to 380 of SEQ. ID NO: 2.

Polynucleotides of the present invention may be in the form of RNA, such as mRNA, or in the form of DNA, including, for instance, cDNA and genomic DNA obtained by cloning or produced by chemical synthetic techniques or by a combination thereof. The DNA may be double-stranded or single-stranded. Single-stranded DNA may be the coding strand, also known as the sense strand, or it may be the non-coding strand, also referred to as the anti-sense strand.

The coding sequence which encodes the polypeptide may be identical to the coding sequence of the polynucleotide shown in Figure 1 (SEQ ID NO: 1). It also may be a polynucleotide with a different sequence, which, as a result of the redundancy (degeneracy) of the genetic code, encodes the polypeptide of the DNA of Figure 1 (SEQ ID NO: 2).

Polynucleotides of the present invention which encode the polypeptide of Figure 1 (SEQ ID NO: 2) may include, but are not limited to the coding sequence for the mature polypeptide, by itself; the coding sequence for the mature polypeptide and additional coding sequences, such as those encoding a leader or secretory sequence, such as a pre-, or pro- or prepro- protein sequence; the coding sequence of the mature polypeptide, with or without the aforementioned additional coding sequences, together with additional, non-coding sequences, including for example, but not limited to introns and non-coding 5' and 3' sequences, such as the transcribed, non-translated sequences that play a role in transcription, mRNA processing - including splicing and polyadenylation signals, for example - ribosome binding and stability of mRNA; additional coding sequence which codes for additional amino acids, such as those which provide additional functionalities. Thus, for instance, the polypeptide may be fused to a marker sequence, such as a peptide, which facilitates purification of the fused polypeptide. In certain preferred embodiments of this aspect of the invention, the marker sequence is a hexa-histidine peptide, such as the tag provided in the pQE vector (Qiagen, Inc.), among others, many of which are commercially available. As described in Gentz et al., Proc. Natl. Acad. Sci., USA 86: 821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. The HA tag corresponds to an epitope derived of influenza hemagglutinin protein, which has been described by Wilson et al., Cell 37: 767 (1984), for instance.

In accordance with the foregoing, the term "polynucleotide encoding a polypeptide" as used herein encompasses polynucleotides which include a sequence encoding a polypeptide of the present invention, particularly the human HR-1 RECEPTOR having the amino acid sequence set out in Figure 1 (SEQ ID NO: 2). The term also encompasses polynucleotides that include a single continuous region or discontinuous regions encoding the polypeptide (for example, interrupted by introns)

together with additional regions, that also may contain coding and/or non-coding sequences.

The present invention further relates to variants of the herein above described polynucleotides which encode for fragments, analogs and derivatives of the polypeptide having the deduced amino acid sequence of Figure 1 (SEQ ID NO: 2). A variant of the polynucleotide may be a naturally occurring variant such as a naturally occurring allelic variant, or it may be a variant that is not known to occur naturally. Such non-naturally occurring variants of the polynucleotide may be made by mutagenesis techniques, including those applied to polynucleotides, cells or organisms.

Among variants in this regard are variants that differ from the aforementioned polynucleotides by nucleotide substitutions, deletions or additions. The substitutions, deletions or additions may involve one or more nucleotides. The variants may be altered in coding or non-coding regions or both. Alterations in the coding regions may produce conservative or non-conservative amino acid substitutions, deletions or additions.

Among the particularly preferred embodiments of the invention in this regard are polynucleotides encoding polypeptides having the amino acid sequence of HR-1 RECEPTOR set out in Figure 1 (SEQ ID NO:2); variants, analogs, derivatives and fragments thereof, and fragments of the variants, analogs and derivatives.

Further particularly preferred in this regard are polynucleotides encoding HR-1 RECEPTOR variants, analogs, derivatives and fragments, and variants, analogs and derivatives of the fragments, which have the amino acid sequence of the HR-1 RECEPTOR polypeptide of Figure 1 (SEQ ID NO:2) in which several, a few, 5 to 10, 1 to 5, 1 to 3, 2, 1 or no amino acid residues are substituted, deleted or added, in any combination. Especially preferred among these are silent substitutions, additions and deletions, which do not alter the properties and activities of the HR-1 RECEPTOR. Also especially preferred in this regard are conservative substitutions. Most highly preferred are polynucleotides encoding polypeptides having the amino acid sequence of Figure 1 (SEQ ID NO:2), without substitutions.

Further preferred embodiments of the invention are polynucleotides that are at least 70% identical to a polynucleotide encoding the HR-1 RECEPTOR polypeptide having the amino acid sequence set out in Figure 1 (SEQ ID NO:2), and polynucleotides which are complementary to such polynucleotides. Alternatively, most highly preferred are polynucleotides that comprise a region that is at least 80% identical to a polynucleotide encoding the HR-1 RECEPTOR polypeptide of the human cDNA of the deposited clone and polynucleotides complementary thereto. In this regard, polynucleotides at least 90% identical to the same are particularly preferred, and among these particularly preferred polynucleotides, those with at least 95% are especially preferred. Furthermore, those with at least 97% are highly preferred among those with at least 95%, and among these those with at least 98% and at least 99% are particularly highly preferred, with at least 99% being the more preferred.

Particularly preferred embodiments in this respect, moreover, are polynucleotides which encode polypeptides which retain substantially the same biological function or activity as the mature polypeptide encoded by the cDNA of Figure 1 (SEQ ID NO: 1).

The present invention further relates to polynucleotides that hybridize to the herein above-described sequences. In this regard, the present invention especially relates to polynucleotides which hybridize under stringent conditions to the herein above-described polynucleotides. As herein used, the term "stringent conditions" means hybridization will occur only if there is at least 95% and preferably at least 97% identity between the sequences.

As discussed additionally herein regarding polynucleotide assays of the invention, for instance, polynucleotides of the invention as discussed above, may be used as a hybridization probe for cDNA and genomic DNA to isolate full-length cDNAs and genomic clones encoding HR-1 RECEPTOR and to isolate cDNA and genomic clones of other genes that have a high sequence similarity to the human HR-1 RECEPTOR gene. Such probes generally will comprise at least 15 bases. Preferably, such probes will have at least 30 bases and may have at least 50 bases. Particularly preferred probes will have at least 30 bases and will have 50 bases or less.

For example, the coding region of the HR-1 RECEPTOR gene may be isolated by screening using the known DNA sequence to synthesize an oligonucleotide probe. A labeled oligonucleotide having a sequence complementary to that of a gene of the present invention is then used to screen a library of human cDNA, genomic DNA or mRNA to determine which members of the library the probe hybridizes to.

The polynucleotides and polypeptides of the present invention may be employed as research reagents and materials for discovery of treatments and diagnostics to human disease, as further discussed herein relating to polynucleotide assays, inter alia.

The polynucleotides may encode a polypeptide which is the mature protein plus additional amino or carboxyl-terminal amino acids, or amino acids interior to the mature polypeptide (when the mature form has more than one polypeptide chain, for instance). Such sequences may play a role in processing of a protein from precursor to a mature form, may facilitate protein trafficking, may prolong or shorten protein half-life or may facilitate manipulation of a protein for assay or production, among other things. As generally is the case *in situ*, the additional amino acids may be processed away from the mature protein by cellular enzymes.

A precursor protein, having the mature form of the polypeptide fused to one or more prosequences may be an inactive form of the polypeptide. When prosequences are removed such inactive precursors generally are activated. Some or all of the prosequences may be removed before activation. Generally, such precursors are called proproteins.

In sum, a polynucleotide of the present invention may encode a mature protein, a mature protein plus a leader sequence (which may be referred to as a preprotein), a precursor of a mature protein having one or more prosequences which are not the leader sequences of a preprotein, or a preproprotein, which is a precursor to a proprotein, having a leader sequence and one or more prosequences, which generally are removed during processing steps that produce active and mature forms of the polypeptide.

Deposited materials

A deposit containing the full length HR-1 RECEPTOR cDNA has been deposited with the American Type Culture Collection, as noted above. Also as noted above, the human cDNA deposit is referred to herein as "the deposited clone" or as "the cDNA of the deposited clone."

The deposited clone was deposited with the American Type Culture Collection, 12301 Park Lawn Drive, Rockville, Maryland 20852, USA, on May 30, 1996, in vector pBluescriptSK$^+$ plasmid (Stratagene, La Jolla, CA), ATG-531, in E. coli JM101 strain, which was assigned ATCC Deposit No. 98069.

The deposit has been made under the terms of the Budapest Treaty on the international recognition of the deposit of micro-organisms for purposes of patent procedure. The strain will be irrevocably and without restriction or condition released to the public upon the issuance of a patent. The deposit is provided merely as convenience to those of skill in the art and is not an admission that a deposit is required for enablement, such as that required under 35 U.S.C. §112.

The sequence of the polynucleotides contained in the deposited material, as well as the amino acid sequence of the polypeptide encoded thereby, are controlling in the event of any conflict with any description of sequences herein.

A license may be required to make, use or sell the deposited materials, and no such license is hereby granted.

Polypeptides

The present invention further relates to a human HR-1 RECEPTOR polypeptide which has the deduced amino acid sequence of Figure 1 (SEQ ID NO:2).

The invention also relates to fragments, analogs and derivatives of these polypeptides. The terms "fragment," "derivative" and "analog" when referring to the polypeptide of Figure 1 (SEQ ID NO:2), means a polypeptide which retains essentially the same biological function or activity as such polypeptide, i.e. functions as a HR-1 RECEPTOR, or retains the ability to bind the ligand or the receptor even though the polypeptide does not function as a HR-1 RECEPTOR, for example, a soluble form of

the receptor. Thus, an analog includes a proprotein which can be activated by cleavage of the proprotein portion to produce an active mature polypeptide.

The polypeptide of the present invention may be a recombinant polypeptide, a natural polypeptide or a synthetic polypeptide. In certain preferred embodiments it is a recombinant polypeptide.

The fragment, derivative or analog of the polypeptide of Figure 1 (SEQ ID NO:2) may be (i) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituent group, or (iii) one in which the mature polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol), or (iv) one in which the additional amino acids are fused to the mature polypeptide, such as a leader or secretory sequence or a sequence which is employed for purification of the mature polypeptide or a proprotein sequence. Such fragments, derivatives and analogs are deemed to be within the scope of those skilled in the art from the teachings herein.

Among the particularly preferred embodiments of the invention in this regard are polypeptides having the amino acid sequence of HR-1 RECEPTOR set out in Figure 1 (SEQ ID NO:2), variants, analogs, derivatives and fragments thereof, and variants, analogs and derivatives of the fragments. Alternatively, particularly preferred embodiments of the invention in this regard are polypeptides having the amino acid sequence of the HR-1 RECEPTOR, variants, analogs, derivatives and fragments thereof, and variants, analogs and derivatives of the fragments.

Among preferred variants are those that vary from a reference by conservative amino acid substitutions. Such substitutions are those that substitute a given amino acid in a polypeptide by another amino acid of like characteristics. Typically seen as conservative substitutions are the replacements, one for another, among the aliphatic amino acids Ala, Val, Leu and Ile; interchange of the hydroxyl residues Ser and Thr, exchange of the acidic residues Asp and Glu, substitution between the amide residues

Asn and Gln, exchange of the basic residues Lys and Arg and replacements among the aromatic residues Phe, Tyr.

Further particularly preferred in this regard are variants, analogs, derivatives and fragments, and variants, analogs and derivatives of the fragments, having the amino acid sequence of the HR-1 RECEPTOR polypeptide of Figure 1 (SEQ ID NO:2), in which several, a few, 5 to 10, 1 to 5, 1 to 3, 2, 1 or no amino acid residues are substituted, deleted or added, in any combination. Especially preferred among these are silent substitutions, additions and deletions, which do not alter the properties and activities of the HR-1 RECEPTOR. Also especially preferred in this regard are conservative substitutions. Most highly preferred are polypeptides having the amino acid sequence of Figure 1 (SEQ ID NO:2) without substitutions.

The polypeptides and polynucleotides of the present invention are preferably provided in an isolated form, and preferably are purified to homogeneity.

The polypeptides of the present invention include the polypeptide of SEQ ID NO:2 (in particular the mature polypeptide) as well as polypeptides which have at least 80% identity to the polypeptide of SEQ ID NO:2 and more preferably at least 90% similarity (more preferably at least 90% identity) to the polypeptide of SEQ ID NO:2 and still more preferably at least 95% similarity (still more preferably at least 95% identity) to the polypeptide of SEQ ID NO:2 and also include portions of such polypeptides with such portion of the polypeptide generally containing at least 30 amino acids and more preferably at least 50 amino acids.

As known in the art "similarity" between two polypeptides is determined by comparing the amino acid sequence and its conserved amino acid substitutes of one polypeptide to the sequence of a second polypeptide. Moreover, also known in the art is "identity" which means the degree of sequence relatedness between two polypptide or two polynucleotides sequences as determined by the identity of the match between two strings of such sequences. Both identity and similarity can be readily calculated (Computational Molecular Biology, Lesk, A.M., ed., Oxford University Press, New York, 1988; Biocomputing: Informatics and Genome Projects, Smith, D.W., ed., Academic Press, New York, 1993; Computer Analysis of Sequence Data, Part I, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994;

Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987; and Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991). While there exist a number of methods to measure identity and similarity between two polynucleotide or polypeptide sequences, the terms "identity" and "similarity" are well known to skilled artisans (Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987; Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991; and Carillo, H., and Lipman, D., SIAM J. Applied Math., 48: 1073 (1988). Methods commonly employed to determine identity or similarity between two sequences include, but are not limited to disclosed in Guide to Huge Computers, Martin J. Bishop, ed., Academic Press, San Diego, 1994, and Carillo, H., and Lipman, D., SIAM J. Applied Math., 48: 1073 (1988). Preferred methods to determine identity are designed to give the largest match between the two sequences tested. Methods to determine identity and similarity are codified in computer programs. Preferred computer program methods to determine identity and similarity between two sequences include, but are not limited to, GCG program package (Devereux, J., et al., *Nucleic Acids Research 12(1):* 387 (1984)), BLASTP, BLASTN, FASTA (Atschul, S.F. et al., *J. Molec. Biol. 215:* 403 (1990)).

Fragments or portions of the polypeptides of the present invention may be employed for producing the corresponding full-length polypeptide by peptide synthesis; therefore, the fragments may be employed as intermediates for producing the full-length polypeptides. Fragments or portions of the polynucleotides of the present invention may be used to synthesize full-length polynucleotides of the present invention.

Fragments

Also among preferred embodiments of this aspect of the present invention are polypeptides comprising fragments of HR-1 RECEPTOR, most particularly fragments of the HR-1 RECEPTOR having the amino acid set out in Figure 1 (SEQ ID NO:2), and fragments of variants and derivatives of the HR-1 RECEPTOR of Figure 1 (SEQ ID NO:2).

In this regard a fragment is a polypeptide having an amino acid sequence that entirely is the same as part but not all of the amino acid sequence of the aforementioned HR-1 RECEPTOR polypeptides and variants or derivatives thereof.

Such fragments may be "free-standing," i.e., not part of or fused to other amino acids or polypeptides, or they may be comprised within a larger polypeptide of which they form a part or region. When comprised within a larger polypeptide, the presently discussed fragments most preferably form a single continuous region. However, several fragments may be comprised within a single larger polypeptide. For instance, certain preferred embodiments relate to a fragment of a HR-1 RECEPTOR polypeptide of the present comprised within a precursor polypeptide designed for expression in a host and having heterologous pre and pro-polypeptide regions fused to the amino terminus of the HR-1 RECEPTOR fragment and an additional region fused to the carboxyl terminus of the fragment. Therefore, fragments in one aspect of the meaning intended herein, refers to the portion or portions of a fusion polypeptide or fusion protein derived from HR-1 RECEPTOR.

As representative examples of polypeptide fragments of the invention, there may be mentioned those which have from about 5-15, 10-20, 15-40, 30-55, 41-75, 41-80, 41-90, 50-100, 75-100, 90-115, 100-125, and 110-113 amino acids long.

In this context about includes the particularly recited range and ranges larger or smaller by several, a few, 5, 4, 3, 2 or 1 amino acid at either extreme or at both extremes. For instance, about 40-90 amino acids in this context means a polypeptide fragment of 40 plus or minus several, a few, 5, 4, 3, 2 or 1 amino acids to 90 plus or minus several a few, 5, 4, 3, 2 or 1 amino acid residues, i.e., ranges as broad as 40 minus several amino acids to 90 plus several amino acids to as narrow as 40 plus several amino acids to 90 minus several amino acids.

Highly preferred in this regard are the recited ranges plus or minus as many as 5 amino acids at either or at both extremes. Particularly highly preferred are the recited ranges plus or minus as many as 3 amino acids at either or at both the recited extremes. Especially particularly highly preferred are ranges plus or minus 1 amino acid at either or at both extremes or the recited ranges with no additions or deletions. Most highly preferred of all in this regard are fragments from about 5-15, 10-20, 15-

40, 30-55, 41-75, 41-80, 41-90, 50-100, 75-100, 90-115, 100-125, and 110-113 amino acids long.

Among especially preferred fragments of the invention are truncation mutants of HR-1 RECEPTOR. Truncation mutants include HR-1 RECEPTOR polypeptides having the amino acid sequence of Figure 1 (SEQ ID NO:2), or of variants or derivatives thereof, except for deletion of a continuous series of residues (that is, a continuous region, part or portion) that includes the amino terminus, or a continuous series of residues that includes the carboxyl terminus or, as in double truncation mutants, deletion of two continuous series of residues, one including the amino terminus and one including the carboxyl terminus. Fragments having the size ranges set out about also are preferred embodiments of truncation fragments, which are especially preferred among fragments generally.

Also preferred in this aspect of the invention are fragments characterized by structural or functional attributes of HR-1 RECEPTOR. Preferred embodiments of the invention in this regard include fragments that comprise alpha-helix and alpha-helix forming regions ("alpha-regions"), beta-sheet and beta-sheet-forming regions ("beta-regions"), turn and turn-forming regions ("turn-regions"), coil and coil-forming regions ("coil-regions"), hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions and high antigenic index regions of HR-1 RECEPTOR.

Among highly preferred fragments in this regard are those that comprise regions of HR-1 RECEPTOR that combine several structural features, such as several of the features set out above. In this regard, the regions defined by the residues about 10 to about 20, about 40 to about 50, about 70 to about 90 and about 100 to about 113 of Figure 1 (SEQ ID NO:2), which all are characterized by amino acid compositions highly characteristic of turn-regions, hydrophilic regions, flexible-regions, surface-forming regions, and high antigenic index-regions, are especially highly preferred regions. Such regions may be comprised within a larger polypeptide or may be by themselves a preferred fragment of the present invention, as discussed above. It will be appreciated that the term "about" as used in this paragraph has the meaning set out above regarding fragments in general.

Further preferred regions are those that mediate activities of HR-1 RECEPTOR. Most highly preferred in this regard are fragments that have a chemical, biological or other activity of HR-1 RECEPTOR, including those with a similar activity or an improved activity, or with a decreased undesirable activity. Highly preferred in this regard are fragments that contain regions that are homologs in sequence, or in position, or in both sequence and to active regions of related polypeptides, such as the related polypeptides which include human IL-5 receptor. Among particularly preferred fragments in these regards are truncation mutants, as discussed above.

It will be appreciated that the invention also relates to, among others, polynucleotides encoding the aforementioned fragments, polynucleotides that hybridize to polynucleotides encoding the fragments, particularly those that hybridize under stringent conditions, and polynucleotides, such as PCR primers, for amplifying polynucleotides that encode the fragments. In these regards, preferred polynucleotides are those that correspondent to the preferred fragments, as discussed above.

Vectors, host cells, expression

The present invention also relates to vectors which include polynucleotides of the present invention, host cells which are genetically engineered with vectors of the invention and the production of polypeptides of the invention by recombinant techniques.

Host cells can be genetically engineered to incorporate polynucleotides and express polypeptides of the present invention. For instance, polynucleotides may be introduced into host cells using well known techniques of infection, transduction, transfection, transvection and transformation. The polynucleotides may be introduced alone or with other polynucleotides. Such other polynucleotides may be introduced independently, co-introduced or introduced joined to the polynucleotides of the invention.

Thus, for instance, polynucleotides of the invention may be transfected into host cells with another, separate, polynucleotide encoding a selectable marker, using standard techniques for co-transfection and selection in, for instance, mammalian cells.

In this case the polynucleotides generally will be stably incorporated into the host cell genome.

Alternatively, the polynucleotides may be joined to a vector containing a selectable marker for propagation in a host. The vector construct may be introduced into host cells by the aforementioned techniques. Generally, a plasmid vector is introduced as DNA in a precipitate, such as a calcium phosphate precipitate, or in a complex with a charged lipid. Electroporation also may be used to introduce polynucleotides into a host. If the vector is a virus, it may be packaged in vitro or introduced into a packaging cell and the packaged virus may be transduced into cells. A wide variety of techniques suitable for making polynucleotides and for introducing polynucleotides into cells in accordance with this aspect of the invention are well known and routine to those of skill in the art. Such techniques are reviewed at length in Sambrook et al. cited above, which is illustrative of the many laboratory manuals that detail these techniques.

In accordance with this aspect of the invention the vector may be, for example, a plasmid vector, a single or double-stranded phage vector, a single or double-stranded RNA or DNA viral vector. Such vectors may be introduced into cells as polynucleotides, preferably DNA, by well known techniques for introducing DNA and RNA into cells. The vectors, in the case of phage and viral vectors also may be and preferably are introduced into cells as packaged or encapsidated virus by well known techniques for infection and transduction. Viral vectors may be replication competent or replication defective. In the latter case viral propagation generally will occur only in complementing host cells.

Preferred among vectors, in certain respects, are those for expression of polynucleotides and polypeptides of the present invention. Generally, such vectors comprise cis-acting control regions effective for expression in a host operatively linked to the polynucleotide to be expressed. Appropriate trans-acting factors either are supplied by the host, supplied by a complementing vector or supplied by the vector itself upon introduction into the host.

In certain preferred embodiments in this regard, the vectors provide for specific expression. Such specific expression may be inducible expression or expression only

in certain types of cells or both inducible and cell-specific. Particularly preferred among inducible vectors are vectors that can be induced for expression by environmental factors that are easy to manipulate, such as temperature and nutrient additives. A variety of vectors suitable to this aspect of the invention, including constitutive and inducible expression vectors for use in prokaryotic and eukaryotic hosts, are well known and employed routinely by those of skill in the art.

The engineered host cells can be cultured in conventional nutrient media, which may be modified as appropriate for, inter alia, activating promoters, selecting transformants or amplifying genes. Culture conditions, such as temperature, pH and the like, previously used with the host cell selected for expression generally will be suitable for expression of polypeptides of the present invention as will be apparent to those of skill in the art.

A great variety of expression vectors can be used to express a polypeptide of the invention. Such vectors include chromosomal, episomal and virus-derived vectors e.g., vectors derived from bacterial plasmids, from bacteriophage, from yeast episomes, from yeast chromosomal elements, from viruses such as baculoviruses, papova viruses, such as SV40, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses and retroviruses, and vectors derived from combinations thereof, such as those derived from plasmid and bacteriophage genetic elements, such as cosmids and phagemids, all may be used for expression in accordance with this aspect of the present invention. Generally, any vector suitable to maintain, propagate or express polynucleotides to express a polypeptide in a host may be used for expression in this regard.

The appropriate DNA sequence may be inserted into the vector by any of a variety of well-known and routine techniques. In general, a DNA sequence for expression is joined to an expression vector by cleaving the DNA sequence and the expression vector with one or more restriction endonucleases and then joining the restriction fragments together using T4 DNA ligase. Procedures for restriction and ligation that can be used to this end are well known and routine to those of skill. Suitable procedures in this regard, and for constructing expression vectors using

alternative techniques, which also are well known and routine to those skilled in the art, are set forth in great detail in Sambrook et al. cited elsewhere herein.

The DNA sequence in the expression vector is operatively linked to appropriate expression control sequence(s), including, for instance, a promoter to direct mRNA transcription. Representatives of such promoters include the phage lambda PL promoter, the E. coli lac, trp and tac promoters, the SV40 early and late promoters and promoters of retroviral LTRs, to name just a few of the well-known promoters. It will be understood that numerous promoters not mentioned are suitable for use in this aspect of the invention are well known and readily may be employed by those of skill in the manner illustrated by the discussion and the examples herein.

In general, expression constructs will contain sites for transcription initiation and termination, and, in the transcribed region, a ribosome binding site for translation. The coding portion of the mature transcripts expressed by the constructs will include a translation initiating AUG at the beginning and a termination codon appropriately positioned at the end of the polypeptide to be translated.

In addition, the constructs may contain control regions that regulate as well as engender expression. Generally, in accordance with many commonly practiced procedures, such regions will operate by controlling transcription, such as repressor binding sites and enhancers, among others.

Vectors for propagation and expression generally will include selectable markers. Such markers also may be suitable for amplification or the vectors may contain additional markers for this purpose. In this regard, the expression vectors preferably contain one or more selectable marker genes to provide a phenotypic trait for selection of transformed host cells. Preferred markers include dihydrofolate reductase or neomycin resistance for eukaryotic cell culture, and tetracycline or ampicillin resistance genes for culturing E. coli and other bacteria.

The vector containing the appropriate DNA sequence as described elsewhere herein, as well as an appropriate promoter, and other appropriate control sequences, may be introduced into an appropriate host using a variety of well known techniques suitable to expression therein of a desired polypeptide. Representative examples of appropriate hosts include bacterial cells, such as E. coli, Streptomyces and Salmonella

typhimurium cells; fungal cells, such as yeast cells; insect cells such as Drosophila S2 and Spodoptera Sf9 cells; animal cells such as CHO, COS and Bowes melanoma cells; and plant cells. Hosts for of a great variety of expression constructs are well known, and those of skill will be enabled by the present disclosure readily to select a host for expressing a polypeptides in accordance with this aspect of the present invention.

More particularly, the present invention also includes recombinant constructs, such as expression constructs, comprising one or more of the sequences described above. The constructs comprise a vector, such as a plasmid or viral vector, into which such a sequence of the invention has been inserted. The sequence may be inserted in a forward or reverse orientation. In certain preferred embodiments in this regard, the construct further comprises regulatory sequences, including, for example, a promoter, operably linked to the sequence. Large numbers of suitable vectors and promoters are known to those of skill in the art, and there are many commercially available vectors suitable for use in the present invention.

The following vectors, which are commercially available, are provided by way of example. Among vectors preferred for use in bacteria are pQE70, pQE60 and pQE-9, available from Qiagen; pBS vectors, Phagescript vectors, Bluescript vectors, pNH8A, pNH16a, pNH18A, pNH46A, available from Stratagene; and ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 available from Pharmacia. Among preferred eukaryotic vectors are pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; and pSVK3, pBPV, pMSG and pSVL available from Pharmacia. These vectors are listed solely by way of illustration of the many commercially available and well known vectors that are available to those of skill in the art for use in accordance with this aspect of the present invention. It will be appreciated that any other plasmid or vector suitable for, for example, introduction, maintenance, propagation or expression of a polynucleotide or polypeptide of the invention in a host may be used in this aspect of the invention.

Promoter regions can be selected from any desired gene using vectors that contain a reporter transcription unit lacking a promoter region, such as a chloramphenicol acetyl transferase ("CAT") transcription unit, downstream of restriction site or sites for introducing a candidate promoter fragment; i.e., a fragment

that may contain a promoter. As is well known, introduction into the vector of a promoter-containing fragment at the restriction site upstream of the cat gene engenders production of CAT activity, which can be detected by standard CAT assays. Vectors suitable to this end are well known and readily available. Two such vectors are pKK232-8 and pCM7. Thus, promoters for expression of polynucleotides of the present invention include not only well known and readily available promoters, but also promoters that readily may be obtained by the foregoing technique, using a reporter gene.

Among known bacterial promoters suitable for expression of polynucleotides and polypeptides in accordance with the present invention are the E. coli lacI and lacZ promoters, the T3 and T7 promoters, the gpt promoter, the lambda PR, PL promoters and the trp promoter.

Among known eukaryotic promoters suitable in this regard are the CMV immediate early promoter, the HSV thymidine kinase promoter, the early and late SV40 promoters, the promoters of retroviral LTRs, such as those of the Rous sarcoma virus ("RSV"), and metallothionein promoters, such as the mouse metallothionein-I promoter.

Selection of appropriate vectors and promoters for expression in a host cell is a well known procedure and the requisite techniques for expression vector construction, introduction of the vector into the host and expression in the host are routine skills in the art.

The present invention also relates to host cells containing the above-described constructs discussed above. The host cell can be a higher eukaryotic cell, such as a mammalian cell, or a lower eukaryotic cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell.

Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection or other methods. Such methods are described in many standard laboratory manuals, such as Davis et al. BASIC METHODS IN MOLECULAR BIOLOGY, (1986).

Constructs in host cells can be used in a conventional manner to produce the gene product encoded by the recombinant sequence. Alternatively, the polypeptides of the invention can be synthetically produced by conventional peptide synthesizers.

Mature proteins can be expressed in mammalian cells, yeast, bacteria, or other cells under the control of appropriate promoters. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention. Appropriate cloning and expression vectors for use with prokaryotic and eukaryotic hosts are described by Sambrook et al., MOLECULAR CLONING: A LABORATORY MANUAL, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989).

Generally, recombinant expression vectors will include origins of replication, a promoter derived from a highly-expressed gene to direct transcription of a downstream structural sequence, and a selectable marker to permit isolation of vector containing cells after exposure to the vector. Among suitable promoters are those derived from the genes that encode glycolytic enzymes such as 3-phosphoglycerate kinase ("PGK"), a-factor, acid phosphatase, and heat shock proteins, among others. Selectable markers include the ampicillin resistance gene of *E. coli* and the trp1 gene of *S. cerevisiae*.

Transcription of the DNA encoding the polypeptides of the present invention by higher eukaryotes may be increased by inserting an enhancer sequence into the vector. Enhancers are cis-acting elements of DNA, usually about from 10 to 300 bp that act to increase transcriptional activity of a promoter in a given host cell-type. Examples of enhancers include the SV40 enhancer, which is located on the late side of the replication origin at bp 100 to 270, the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers.

Polynucleotides of the invention, encoding the heterologous structural sequence of a polypeptide of the invention generally will be inserted into the vector using standard techniques so that it is operably linked to the promoter for expression. The polynucleotide will be positioned so that the transcription start site is located appropriately 5' to a ribosome binding site. The ribosome binding site will be 5' to the AUG that initiates translation of the polypeptide to be expressed. Generally, there will

be no other open reading frames that begin with an initiation codon, usually AUG, and lie between the ribosome binding site and the initiating AUG. Also, generally, there will be a translation stop codon at the end of the polypeptide and there will be a polyadenylation signal and a transcription termination signal appropriately disposed at the 3' end of the transcribed region.

For secretion of the translated protein into the lumen of the endoplasmic reticulum, into the periplasmic space or into the extracellular environment, appropriate secretion signals may be incorporated into the expressed polypeptide. The signals may be endogenous to the polypeptide or they may be heterologous signals.

The polypeptide may be expressed in a modified form, such as a fusion protein, and may include not only secretion signals but also additional heterologous functional regions. Thus, for instance, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the polypeptide to improve stability and persistence in the host cell, during purification or during subsequent handling and storage. Also, region also may be added to the polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the polypeptide. The addition of peptide moieties to polypeptides to engender secretion or excretion, to improve stability and to facilitate purification, among others, are familiar and routine techniques in the art.

Suitable prokaryotic hosts for propagation, maintenance or expression of polynucleotides and polypeptides in accordance with the invention include Escherichia coli, Bacillus subtilis and Salmonella typhimurium. Various species of Pseudomonas, Streptomyces, and Staphylococcus are suitable hosts in this regard. Moreover, many other hosts also known to those of skill may be employed in this regard.

As a representative but non-limiting example, useful expression vectors for bacterial use can comprise a selectable marker and bacterial origin of replication derived from commercially available plasmids comprising genetic elements of the well known cloning vector pBR322 (ATCC 37017). Such commercial vectors include, for example, pKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden) and GEM1 (Promega Biotec, Madison, WI, USA). These pBR322 "backbone" sections are combined with an appropriate promoter and the structural sequence to be expressed.

Following transformation of a suitable host strain and growth of the host strain to an appropriate cell density, where the selected promoter is inducible it is induced by appropriate means (e.g., temperature shift or exposure to chemical inducer) and cells are cultured for an additional period.

Cells typically then are harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification.

Microbial cells employed in expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents, such methods are well know to those skilled in the art.

Various mammalian cell culture systems can be employed for expression, as well. Examples of mammalian expression systems include the COS-7 lines of monkey kidney fibroblast, described in Gluzman et al., Cell 23: 175 (1981). Other cell lines capable of expressing a compatible vector include for example, the C127, 3T3, CHO, HeLa, human kidney 293 and BHK cell lines.

Mammalian expression vectors will comprise an origin of replication, a suitable promoter and enhancer, and also any necessary ribosome binding sites, polyadenylation sites, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking non-transcribed sequences that are necessary for expression. In certain preferred embodiments in this regard DNA sequences derived from the SV40 splice sites, and the SV40 polyadenylation sites are used for required non-transcribed genetic elements of these types.

The HR-1 RECEPTOR polypeptide can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography ("HPLC") is employed for purification. Well known techniques for refolding protein may be employed to regenerate active conformation when the polypeptide is denatured during isolation and or purification.

Polypeptides of the present invention include naturally purified products, products of chemical synthetic procedures, and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect and mammalian cells. Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. In addition, polypeptides of the invention may also include an initial modified methionine residue, in some cases as a result of host-mediated processes.

HR-1 RECEPTOR polynucleotides and polypeptides may be used in accordance with the present invention for a variety of applications, particularly those that make use of the chemical and biological properties of HR-1 RECEPTOR. Additional applications relate to diagnosis and to treatment of disorders of cells, tissues and organisms. These aspects of the invention are illustrated further by the following discussion.

Polynucleotide assays

This invention is also related to the use of the HR-1 RECEPTOR polynucleotides to detect complementary polynucleotides such as, for example, as a diagnostic reagent. Detection of a mutated form of HR-1 RECEPTOR associated with a dysfunction will provide a diagnostic tool that can add or define a diagnosis of a disease or susceptibility to a disease which results from under-expression over-expression or altered expression of HR-1 RECEPTOR. Individuals carrying mutations in the human HR-1 RECEPTOR gene may be detected at the DNA level by a variety of techniques. Nucleic acids for diagnosis may be obtained from a patient's cells, such as from blood, urine, saliva, tissue biopsy and autopsy material. The genomic DNA may be used directly for detection or may be amplified enzymatically by using PCR prior to analysis. PCR (Saiki et al., Nature, 324: 163-166 (1986)). RNA or cDNA may also be used in the same ways. As an example, PCR primers complementary to the nucleic acid encoding HR-1 RECEPTOR can be used to identify and analyze HR-1 RECEPTOR expression and mutations. For example, deletions and insertions can be detected by a change in size of the amplified product in comparison to the normal

genotype. Point mutations can be identified by hybridizing amplified DNA to radiolabeled HR-1 RECEPTOR RNA or alternatively, radiolabeled HR-1 RECEPTOR antisense DNA sequences. Perfectly matched sequences can be distinguished from mismatched duplexes by RNase A digestion or by differences in melting temperatures.

Sequence differences between a reference gene and genes having mutations also may be revealed by direct DNA sequencing. In addition, cloned DNA segments may be employed as probes to detect specific DNA segments. The sensitivity of such methods can be greatly enhanced by appropriate use of PCR or another amplification method. For example, a sequencing primer is used with double-stranded PCR product or a single-stranded template molecule generated by a modified PCR. The sequence determination is performed by conventional procedures with radiolabeled nucleotide or by automatic sequencing procedures with fluorescent-tags.

Genetic testing based on DNA sequence differences may be achieved by detection of alteration in electrophoretic mobility of DNA fragments in gels, with or without denaturing agents. Small sequence deletions and insertions can be visualized by high resolution gel electrophoresis. DNA fragments of different sequences may be distinguished on denaturing formamide gradient gels in which the mobilities of different DNA fragments are retarded in the gel at different positions according to their specific melting or partial melting temperatures (see, e.g., Myers et al., Science, 230: 1242 (1985)).

Sequence changes at specific locations also may be revealed by nuclease protection assays, such as RNase and S1 protection or the chemical cleavage method (e.g., Cotton et al., Proc. Natl. Acad. Sci., USA, 85: 4397-4401 (1985)).

Thus, the detection of a specific DNA sequence may be achieved by methods such as hybridization, RNase protection, chemical cleavage, direct DNA sequencing or the use of restriction enzymes, (e.g., restriction fragment length polymorphisms ("RFLP") and Southern blotting of genomic DNA.

In addition to more conventional gel-electrophoresis and DNA sequencing, mutations also can be detected by *in situ* analysis.

In accordance with a further aspect of the invention, there is provided a process for determining decreased level of resistance to infection, asthma, various allergic and

hematopoietic disorders or a susceptibility to the aforementioned abnormalities. Thus, a mutation in HR-1 RECEPTOR indicates a susceptibility to decreased level of infection, asthma, various allergic and hematopoietic disorders, and the nucleic acid sequences described above may be employed in an assay for ascertaining such susceptibility. Thus, for example, the assay may be employed to determine a mutation in a human HR-1 RECEPTOR protein as herein described, such as a deletion, truncation, insertion, frame shift, etc., with such mutation being indicative of a susceptibility to decreased level resistance to infection, asthma, various allergic and hematopoietic disorders.

A mutation may be ascertained for example, by a DNA sequencing assay. Tissue samples, including but not limited to blood samples are obtained from a human patient. The samples are processed by methods known in the art to capture the RNA. First strand cDNA is synthesized from the RNA samples by adding an oligonucleotide primer consisting of polythymidine residues which hybridize to the polyadenosine stretch present on the mRNA's. Reverse transcriptase and deoxynucleotides are added to allow synthesis of the first strand cDNA. Primer sequences are synthesized based on the DNA sequence of the DNA repair protein of the invention. The primer sequence is generally comprised of at least 15 consecutive bases, and may contain at least 30 or even 50 consecutive bases.

Individuals carrying mutations in the gene of the present invention may also be detected at the DNA level by a variety of techniques. Nucleic acids for diagnosis may be obtained from a patient's cells, including but not limited to blood, urine, saliva, tissue biopsy and autopsy material. The genomic DNA may be used directly for detection or may be amplified enzymatically by using PCR (Saiki *et al.*, Nature, 324:163-166 (1986)) prior to analysis. RT-PCR can also be used to detect mutations. It is particularly preferred to used RT-PCR in conjunction with automated detection systems, such as, for example, GeneScan. RNA or cDNA may also be used for the same purpose, PCR or RT-PCR. As an example, PCR primers complementary to the nucleic acid encoding HR-1 RECEPTOR can be used to identify and analyze mutations. For example, deletions and insertions can be detected by a change in size of the amplified product in comparison to the normal genotype. Point mutations can

be identified by hybridizing amplified DNA to radiolabeled RNA or alternatively, radiolabeled antisense DNA sequences. Perfectly matched sequences can be distinguished from mismatched duplexes by RNase A digestion or by differences in melting temperatures.

The primers may be used for amplifying HR-1 RECEPTOR cDNA isolated from a sample derived from a patient. The invention also provides the primers with 1, 2, 3 or 4 nucleotides removed from the 5' and/or the 3' end. The primers may be used to amplify the gene isolated from the patient such that the gene may then be subject to various techniques for elucidation of the DNA sequence. In this way, mutations in the DNA sequence may be diagnosed. The primers that can be used in this connection are obvious to the skilled in the art.

Sequence differences between the reference gene and genes having mutations may be revealed by the direct DNA sequencing method. In addition, cloned DNA segments may be employed as probes to detect specific DNA segments. The sensitivity of this method is greatly enhanced when combined with PCR. For example, a sequencing primer is used with double-stranded PCR product or a single-stranded template molecule generated by a modified PCR. The sequence determination is performed by conventional procedures with radiolabeled nucleotide or by automatic sequencing procedures with fluorescent-tags.

Genetic testing based on DNA sequence differences may be achieved by detection of alteration in electrophoretic mobility of DNA fragments in gels with or without denaturing agents. Small sequence deletions and insertions can be visualized by high resolution gel electrophoresis. DNA fragments of different sequences may be distinguished on denaturing formamide gradient gels in which the mobilities of different DNA fragments are retarded in the gel at different positions according to their specific melting or partial melting temperatures (see, e.g., Myers *et al.*, Science, 230:1242 (1985)).

Sequence changes at specific locations may also be revealed by nuclease protection assays, such as RNase and S1 protection or the chemical cleavage method (e.g., Cotton *et al.*, PNAS, USA, 85:4397-4401 (1985)).

Thus, the detection of a specific DNA sequence and/or quantitation of the level of the sequence may be achieved by methods such as hybridization, RNase protection, chemical cleavage, direct DNA sequencing or the use of restriction enzymes, (e.g., Restriction Fragment Length Polymorphisms (RFLP)) and Southern blotting of genomic DNA. The invention provides a process for diagnosing, disease, particularly decreased level of resistance to infection, asthma, various allergic and hematopoietic disorders, comprising determining from a sample derived from a patient a decreased level of expression of polynucleotide having the sequence of Figure 1 (SEQ ID NO: 1). Decreased expression of polynucleotide can be measured using any on of the methods well known in the art for the quantation of polynucleotides, such as, for example, PCR, RT-PCR, RNase protection, Northern blotting and other hybridization methods.

In addition to more conventional gel-electrophoresis and DNA sequencing, mutations can also be detected by *in situ* analysis.

Fluorescence *in situ* hybridization (FISH) of a cDNA clone to a metaphase chromosomal spread can be used to provide a precise chromosomal location.

As an example of how this is performed, HR-1 RECEPTOR DNA is digested and purified with QIAEX II DNA purification kit (QIAGEN, Inc., Chatsworth, CA) and ligated to Super Cos1 cosmid vector (STRATAGENE, La Jolla, CA). DNA is purified using Qiagen Plasmid Purification Kit (QIAGEN Inc., Chatsworth, CA) and 1 mg is labeled by nick translation in the presence of Biotin-dATP using BioNick Labeling Kit (GibcoBRL, Life Technologies Inc., Gaithersburg, MD). Biotinilation is detected with GENE-TECT Detection System (CLONTECH Laboratories, Inc. Palo Alto, CA). *In situ* Hybridization is performed on slides using ONCOR Light Hybridization Kit (ONCOR, Gaithersberg, MD) to detect single copy sequences on metaphase chromosomes. Peripheral blood of normal donors is cultured for three days in RPMI 1640 supplemented with 20% FCS, 3% PHA and penicillin/ streptomycin, synchronized with 10^{-7} M methotrexate for 17 hours and washed twice with unsupplemented RPMI. Cells are incubated with 10^{-3} M thymidine for 7 hours. The cells are arrested in metaphase after 20 minutes incubation with colcemid (0.5 µg/ml)

followed by hypotonic lysis in 75 mM KCl for 15 minutes at 37°C. Cell pellets are then spun out and fixed in Carnoy's fixative (3:1 methanol/acetic acid).

Metaphase spreads are prepared by adding a drop of the suspension onto slides and aid dried. Hybridization is performed by adding 100 ng of probe suspended in 10 ml of hybridization mix (50% formamide, 2xSSC, 1% dextran sulfate) with blocking human placental DNA 1 µg/ml), Probe mixture is denatured for 10 minutes in 70°C water bath and incubated for 1 hour at 37°C, before placing on a prewarmed (37°C) slide, which is previously denatured in 70% formamide/2xSSC at 70°C, and dehydrated in ethanol series, chilled to 4°C.

Slides are incubated for 16 hours at 37°C in a humidified chamber. Slides are washed in 50% formamide/2xSSC for 10 minutes at 41°C and 2xSSC for 7 minutes at 37°C. Hybridization probe is detected by incubation of the slides with FITC-Avidin (ONCOR, Gaithersberg, MD), according to the manufacturer protocol. Chromosomes are counterstained with propridium iodine suspended in mounting medium. Slides are visualized using a Leitz ORTHOPLAN 2-epifluorescence microscope and five computer images are taken using Imagenetics Computer and MacIntosh printer.

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, for example, in V. McKusick, Mendelian Inheritance in Man, which is publicly available on line via computer. The relationship between genes and diseases that have been mapped to the same chromosomal region are then identified through linkage analysis (Co-Inheritance of Physically Adjacent Genes).

Unless otherwise stated, transformation was performed as described in the method of Graham, F. and Van der Eb, A., Virology, 52:456-457 (1973).

Chromosome assays

The sequences of the present invention are also valuable for chromosome identification. The sequence is specifically targeted to and can hybridize with a particular location on an individual human chromosome. Moreover, there is a current need for identifying particular sites on the chromosome. Few chromosome marking reagents based on actual sequence data (repeat polymorphisms) are presently available

for marking chromosomal location. The mapping of DNAs to chromosomes according to the present invention is an important first step in correlating those sequences with genes associated with disease.

In certain preferred embodiments in this regard, the cDNA herein disclosed is used to clone genomic DNA of a HR-1 RECEPTOR gene. This can be accomplished using a variety of well known techniques and libraries, which generally are available commercially. The genomic DNA the is used for *in situ* chromosome mapping using well known techniques for this purpose. Typically, in accordance with routine procedures for chromosome mapping, some trial and error may be necessary to identify a genomic probe that gives a good *in situ* hybridization signal.

In some cases, in addition, sequences can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp) from the cDNA. Computer analysis of the 3' untranslated region of the gene is used to rapidly select primers that do not span more than one exon in the genomic DNA, thus complicating the amplification process. These primers are then used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the primer will yield an amplified fragment.

PCR mapping of somatic cell hybrids is a rapid procedure for assigning a particular DNA to a particular chromosome. Using the present invention with the same oligonucleotide primers, sublocalization can be achieved with panels of fragments from specific chromosomes or pools of large genomic clones in an analogous manner. Other mapping strategies that can similarly be used to map to its chromosome include *in situ* hybridization, prescreening with labeled flow-sorted chromosomes and preselection by hybridization to construct chromosome specific-cDNA libraries.

Fluorescence *in situ* hybridization ("FISH") of a cDNA clone to a metaphase chromosomal spread can be used to provide a precise chromosomal location in one step. This technique can be used with cDNA as short as 50 or 60. For a review of this technique, see Verma et al., HUMAN CHROMOSOMES: A MANUAL OF BASIC TECHNIQUES, Pergamon Press, New York (1988).

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic

map data. Such data are found, for example, in V. McKusick, MENDELIAN INHERITANCE IN MAN, available on line through Johns Hopkins University, Welch Medical Library. The relationship between genes and diseases that have been mapped to the same chromosomal region are then identified through linkage analysis (coinheritance of physically adjacent genes).

Next, it is necessary to determine the differences in the cDNA or genomic sequence between affected and unaffected individuals. If a mutation is observed in some or all of the affected individuals but not in any normal individuals, then the mutation is likely to be the causative agent of the disease.

With current resolution of physical mapping and genetic mapping techniques, a cDNA precisely localized to a chromosomal region associated with the disease could be one of between 50 and 500 potential causative genes. (This assumes 1 megabase mapping resolution and one gene per 20 kb).

Polypeptide assays

The present invention also relates to a diagnostic assays such as quantitative and diagnostic assays for detecting levels of HR-1 RECEPTOR protein or ligands in cells and tissues, including determination of normal and abnormal levels. Thus, for instance, a diagnostic assay in accordance with the invention for detecting over-expression of HR-1 RECEPTOR protein compared to normal control tissue samples may be used to detect the presence of a tumor, for example. Assay techniques that can be used to determine levels of a protein, such as an HR-1 RECEPTOR protein of the present invention, in a sample derived from a host are well-known to those of skill in the art. Such assay methods include radioimmunoassays, competitive-binding assays, Western Blot analysis and ELISA assays. Among these ELISAs frequently are preferred. An ELISA assay initially comprises preparing an antibody specific to HR-1 RECEPTOR, preferably a monoclonal antibody. In addition a reporter antibody generally is prepared which binds to the monoclonal antibody. The reporter antibody is attached a detectable reagent such as radioactive, fluorescent or enzymatic reagent, in this example horseradish peroxidase enzyme.

To carry out an ELISA a sample is removed from a host and incubated on a solid support, e.g. a polystyrene dish, that binds the proteins in the sample. Any free protein binding sites on the dish are then covered by incubating with a non-specific protein such as bovine serum albumin. Next, the monoclonal antibody is incubated in the dish during which time the monoclonal antibodies attach to any HR-1 RECEPTOR proteins attached to the polystyrene dish. Unbound monoclonal antibody is washed out with buffer. The reporter antibody linked to horseradish peroxidase is placed in the dish resulting in binding of the reporter antibody to any monoclonal antibody bound to HR-1 RECEPTOR. Unattached reporter antibody is then washed out. Reagents for peroxidase activity, including a colorimetric substrate are then added to the dish. Immobilized peroxidase, linked to HR-1 RECEPTOR through the primary and secondary antibodies, produces a colored reaction product. The amount of color developed in a given time period indicates the amount of HR-1 RECEPTOR protein present in the sample. Quantitative results typically are obtained by reference to a standard curve.

A competition assay may be employed wherein antibodies specific to HR-1 RECEPTOR attached to a solid support and labeled HR-1 RECEPTOR and a sample derived from the host are passed over the solid support and the amount of label detected attached to the solid support can be correlated to a quantity of HR-1 RECEPTOR in the sample.

Antibodies

The polypeptides, their fragments or other derivatives, or analogs thereof, or cells expressing them can be used as an immunogen to produce antibodies thereto. These antibodies can be, for example, polyclonal or monoclonal antibodies. The present invention also includes chimeric, single chain, and humanized antibodies, as well as Fab fragments, or the product of an Fab expression library. Various procedures known in the art may be used for the production of such antibodies and fragments.

Antibodies generated against the polypeptides corresponding to a sequence of the present invention can be obtained by direct injection of the polypeptides into an animal or by administering the polypeptides to an animal, preferably a nonhuman. The

antibody so obtained will then bind the polypeptides itself. In this manner, even a sequence encoding only a fragment of the polypeptides can be used to generate antibodies binding the whole native polypeptides. Such antibodies can then be used to isolate the polypeptide from tissue expressing that polypeptide.

For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique (Kohler, G. and Milstein, C., Nature 256: 495-497 (1975), the trioma technique, the human B-cell hybridoma technique (Kozbor et al., Immunology Today 4: 72 (1983) and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole et al., pg. 77-96 in MONOCLONAL ANTIBODIES AND CANCER THERAPY, Alan R. Liss, Inc. (1985).

Techniques described for the production of single chain antibodies (U.S. Patent No. 4,946,778) can be adapted to produce single chain antibodies to immunogenic polypeptide products of this invention. Also, transgenic mice, or other organisms such as other mammals, may be used to express humanized antibodies to immunogenic polypeptide products of this invention.

The above-described antibodies may be employed to isolate or to identify clones expressing the polypeptide or purify the polypeptide of the present invention by attachment of the antibody to a solid support for isolation and/or purification by affinity chromatography.

Thus, among others, antibodies against HR-1 RECEPTOR may be employed to inhibit decreased level of resistance to infection, asthma, various allergic and hematopoietic disorders.

HR-1 RECEPTOR may also be employed to inhibit or treat decreased level of resistance to infection, asthma, various allergic and hematopoietic disorders.

HR-1 RECEPTOR binding molecules and assays

This invention also provides a method for identification of molecules, such as receptor molecules, that bind HR-1 RECEPTOR. Genes encoding proteins that bind HR-1 RECEPTOR, such as receptor proteins, can be identified by numerous methods known to those of skill in the art. Such methods are described in many laboratory manuals such as, for instance, Coligan et al., Current Protocols in Immunology 1(2): Chapter 5 (1991).

Polypeptides of the invention also can be used to assess HR-1 RECEPTOR binding capacity of HR-1 RECEPTOR binding molecules, such as receptor molecules, in cells or in cell-free preparations.

The HR-1 RECEPTOR of the present invention may be employed in a process for screening for compounds which activate (agonists) or inhibit activation (antagonists) of the receptor polypeptide of the present invention .

In general, such screening procedures involve providing appropriate cells which express the receptor polypeptide of the present invention on the surface thereof. Such cells include cells from mammals, yeast, drosophila or *E. Coli*. In particular, a polynucleotide encoding the receptor of the present invention is employed to transfect cells to thereby express the HR-1 RECEPTOR. The expressed receptor is then contacted with a test compound to observe binding, stimulation or inhibition of a functional response.

The screen may be employed for determining a compound which activates the receptor by contacting such cells with compounds to be screened and determining whether such compound generates a signal, i.e., activates the receptor.

Other screening techniques include the use of cells which express the HR-1 RECEPTOR (for example, transfected CHO cells) in a system which measures intracellular pH changes caused by receptor activation, for example, as described in Science, volume 246, pages 181-296 (October 1989). For example, compounds may be contacted with a cell which expresses the receptor polypeptide of the present invention and a second messenger response, e.g. signal transduction or pH changes, may be measured to determine whether the potential compound activates or inhibits the receptor.

Another such screening technique involves transfecting HR-1 RECEPTOR gene into a cell line and look for ligands which cause phosphorylation events.

Another method involves screening for compounds which inhibit activation of the receptor polypeptide of the present invention antagonists by determining inhibition of binding of labeled ligand to cells which have the receptor on the surface thereof. Such a method involves transfecting a eukaryotic cell with DNA encoding the HR-1 RECEPTOR such that the cell expresses the receptor on its surface and contacting the cell with a compound in the presence of a labeled form of a known ligand. The ligand can be labeled, e.g., by radioactivity. The amount of labeled ligand bound to the receptors is measured, e.g., by measuring radioactivity of the receptors. If the compound binds to the receptor as determined by a reduction of labeled ligand which binds to the receptors, the binding of labeled ligand to the receptor is inhibited.

HR-1 RECEPTORS in the mammalian host and are responsible for many biological functions, including many pathologies. Accordingly, it is desirous to find compounds and drugs which stimulate the HR-1 RECEPTOR on the one hand and which can inhibit the function of a HR-1 RECEPTOR on the other hand.

For example, compounds which activate the HR-1 RECEPTOR may be employed for therapeutic purposes, such as the treatment of decreased level of resistance to infection, asthma, various allergic and hematopoietic disorders.

In general, compounds which inhibit activation of the HR-1 RECEPTOR may be employed for a variety of therapeutic purposes, for example, for the treatment of decreased level of resistance to infection, asthma, various allergic and hematopoietic disorders, among others. Compounds which inhibit HR-1 RECEPTOR have also been useful in reversing decreased level of resistance to infection, asthma, various allergic and hematopoietic disorders.

An antibody may antagonize a HR-1 RECEPTOR of the present invention, or in some cases an oligopeptide, which bind to the HR-1 RECEPTOR but does not elicit a second messenger response such that the activity of the HR-1 RECEPTOR is prevented. Antibodies include anti-idiotypic antibodies which recognize unique determinants generally associated with the antigen-binding site of an antibody. Potential antagonist compounds also include proteins which are closely related to the

ligand of the HR-1 RECEPTOR, i.e. a fragment of the ligand, which have lost biological function and when binding to the HR-1 RECEPTOR, elicit no response.

An antisense construct prepared through the use of antisense technology, may be used to control gene expression through triple-helix formation or antisense DNA or RNA, both of which methods are based on binding of a polynucleotide to DNA or RNA. For example, the 5' coding portion of the polynucleotide sequence, which encodes for the mature polypeptides of the present invention, is used to design an antisense RNA oligonucleotide of from about 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription (triple helix -see Lee et al., *Nucl. Acids Res.*, *6:*3073 (1979); Cooney et al, Science, 241:456 (1988); and Dervan et al., *Science, 251:* 1360 (1991)), thereby preventing transcription and the production of HR-1 RECEPTOR. The antisense RNA oligonucleotide hybridizes to the mRNA *in vivo* and blocks translation of mRNA molecules into HR-1 RECEPTOR (antisense - Okano, *J. Neurochem.*, *56:*560 (1991); Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988)). The oligonucleotides described above can also be delivered to cells such that the antisense RNA or DNA may be expressed *in vivo* to inhibit production of HR-1 RECEPTOR.

A small molecule which binds to the HR-1 RECEPTOR, making it inaccessible to ligands such that normal biological activity is prevented, for example small peptides or peptide-like molecules, may also be used to inhibit activation of the receptor polypeptide of the present invention.

A soluble form of the HR-1 RECEPTOR, e.g. a fragment of the receptors, may be used to inhibit activation of the receptor by binding to the ligand to a polypeptide of the present invention and preventing the ligand from interacting with membrane bound HR-1 RECEPTOR.

This invention additionally provides a method of treating an abnormal condition related to an excess of HR-1 RECEPTOR activity which comprises administering to a subject the inhibitor compounds as hereinabove described along with a pharmaceutically acceptable carrier in an amount effective to inhibit activation

by blocking binding of ligands to the HR-1 RECEPTOR, or by inhibiting a second signal, and thereby alleviating the abnormal conditions.

The invention also provides a method of treating abnormal conditions related to an under-expression of HR-1 RECEPTOR activity which comprises administering to a subject a therapeutically effective amount of a compound which activates the receptor polypeptide of the present invention as described above in combination with a pharmaceutically acceptable carrier, to thereby alleviate the abnormal conditions.

The soluble form of the HR-1 RECEPTOR, and compounds which activate or inhibit such receptor, may be employed in combination with a suitable pharmaceutical carrier. Such compositions comprise a therapeutically effective amount of the polypeptide or compound, and a pharmaceutically acceptable carrier or excipient. Such a carrier includes but is not limited to saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. The formulation should suit the mode of administration.

Agonists and antagonists - assays and molecules

The invention also provides a method of screening compounds to identify those which enhance or block the action of HR-1 RECEPTOR on cells, such as its interaction with HR-1 RECEPTOR-binding molecules such as receptor molecules. An agonist is a compound which increases the natural biological functions of HR-1 RECEPTOR or which functions in a manner similar to HR-1 RECEPTOR, while antagonists decrease or eliminate such functions.

For example, a cellular compartment, such as a membrane or a preparation thereof, such as a membrane-preparation, may be prepared from a cell that expresses a molecule that binds HR-1 RECEPTOR, such as a molecule of a signaling or regulatory pathway modulated by HR-1 RECEPTOR. The preparation is incubated with labeled HR-1 RECEPTOR in the absence or the presence of a candidate molecule which may be a HR-1 RECEPTOR agonist or antagonist. The ability of the candidate molecule to bind the binding molecule is reflected in decreased binding of the labeled ligand. Molecules which bind gratuitously, i.e., without inducing the effects of HR-1 RECEPTOR on binding the HR-1 RECEPTOR binding molecule, are most likely to

be good antagonists. Molecules that bind well and elicit effects that are the same as or closely related to HR-1 RECEPTOR are agonists.

HR-1 RECEPTOR-like effects of potential agonists and antagonists may by measured, for instance, by determining activity of a second messenger system following interaction of the candidate molecule with a cell or appropriate cell preparation, and comparing the effect with that of HR-1 RECEPTOR or molecules that elicit the same effects as HR-1 RECEPTOR. Second messenger systems that may be useful in this regard include but are not limited to AMP guanylate cyclase, ion channel or phosphoinositide hydrolysis second messenger systems.

Another example of an assay for HR-1 RECEPTOR antagonists is a competitive assay that combines HR-1 RECEPTOR and a potential antagonist with membrane-bound HR-1 RECEPTOR receptor molecules or recombinant HR-1 RECEPTOR receptor molecules under appropriate conditions for a competitive inhibition assay. HR-1 RECEPTOR can be labeled, such as by radioactivity, such that the number of HR-1 RECEPTOR molecules bound to a receptor molecule can be determined accurately to assess the effectiveness of the potential antagonist.

Potential antagonists include small organic molecules, peptides, polypeptides and antibodies that bind to a polypeptide of the invention and thereby inhibit or extinguish its activity. Potential antagonists also may be small organic molecules, a peptide, a polypeptide such as a closely related protein or antibody that binds the same sites on a binding molecule, such as a receptor molecule, without inducing HR-1 RECEPTOR-induced activities, thereby preventing the action of HR-1 RECEPTOR by excluding HR-1 RECEPTOR from binding.

Potential antagonists include a small molecule which binds to and occupies the binding site of the polypeptide thereby preventing binding to cellular binding molecules, such as receptor molecules, such that normal biological activity is prevented. Examples of small molecules include but are not limited to small organic molecules, peptides or peptide-like molecules.

Other potential antagonists include antisense molecules. Antisense technology can be used to control gene expression through antisense DNA or RNA or through triple-helix formation. Antisense techniques are discussed, for example, in - Okano, J.

Neurochem. 56: 560 (1991); OLIGODEOXYNUCLEOTIDES AS ANTISENSE INHIBITORS OF GENE EXPRESSION, CRC Press, Boca Raton, FL (1988). Triple helix formation is discussed in, for instance Lee et al., Nucleic Acids Research 6: 3073 (1979); Cooney et al., Science 241: 456 (1988); and Dervan et al., Science 251: 1360 (1991). The methods are based on binding of a polynucleotide to a complementary DNA or RNA. For example, the 5' coding portion of a polynucleotide that encodes the mature polypeptide of the present invention may be used to design an antisense RNA oligonucleotide of from about 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription thereby preventing transcription and the production of HR-1 RECEPTOR. The antisense RNA oligonucleotide hybridizes to the mRNA in vivo and blocks translation of the mRNA molecule into HR-1 RECEPTOR polypeptide. The oligonucleotides described above can also be delivered to cells such that the antisense RNA or DNA may be expressed in vivo to inhibit production of HR-1 RECEPTOR.

Potential agonists include small organic molecules, peptides, peptide-like molecules, polypeptides and antibodies that bind to a polypeptide of the invention and thereby elicit its activity. Potential agonists also may be small organic molecules, a peptide, a polypeptide such as a closely related protein or antibody that binds the same sites on a binding molecule, such as a receptor molecule, inducing HR-1 RECEPTOR activities.

The antagonists and/or agonists may be employed in a composition with a pharmaceutically acceptable carrier, e.g., as hereinafter described.

The antagonists and/or agonists may be employed for instance to inhibit decreased level of resistance to infection, asthma, various allergic and hematopoietic disorders.

Compositions

The invention also relates to compositions comprising the polynucleotide or the polypeptides discussed above or the agonists or antagonists. Thus, the polypeptides of the present invention may be employed in combination with a non-sterile or sterile carrier or carriers for use with cells, tissues or organisms, such as a pharmaceutical carrier suitable for administration to a subject. Such compositions

comprise, for instance, a media additive or a therapeutically effective amount of a polypeptide of the invention and a pharmaceutically acceptable carrier or excipient. Such carriers may include, but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol and combinations thereof. The formulation should suit the mode of administration.

Kits

The invention further relates to pharmaceutical packs and kits comprising one or more containers filled with one or more of the ingredients of the aforementioned compositions of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, reflecting approval by the agency of the manufacture, use or sale of the product for human administration.

Administration

Polypeptides and other compounds of the present invention may be employed alone or in conjunction with other compounds, such as therapeutic compounds.

The pharmaceutical compositions may be administered in any effective, convenient manner including, for instance, administration by topical, oral, anal, vaginal, intravenous, intraperitoneal, intramuscular, subcutaneous, intranasal or intradermal routes among others.

The pharmaceutical compositions generally are administered in an amount effective for treatment or prophylaxis of a specific indication or indications. In general, the compositions are administered in an amount of at least about 10 μg/kg body weight. In most cases they will be administered in an amount not in excess of about 8 mg/kg body weight per day. Preferably, in most cases, dose is from about 10 μg/kg to about 1 mg/kg body weight, daily. It will be appreciated that optimum dosage will be determined by standard methods for each treatment modality and indication, taking into account the indication, its severity, route of administration, complicating conditions and the like.

Gene therapy

The HR-1 RECEPTOR polynucleotides, polypeptides, agonists and antagonists that are polypeptides may be employed in accordance with the present invention by expression of such polypeptides *in vivo*, in treatment modalities often referred to as "gene therapy."

Thus, for example, cells from a patient may be engineered with a polynucleotide, such as a DNA or RNA, encoding a polypeptide ex vivo, and the engineered cells then can be provided to a patient to be treated with the polypeptide. For example, cells may be engineered ex vivo by the use of a retroviral plasmid vector containing RNA encoding a polypeptide of the present invention. Such methods are well-known in the art and their use in the present invention will be apparent from the teachings herein.

Similarly, cells may be engineered *in vivo* for expression of a polypeptide *in vivo* by procedures known in the art. For example, a polynucleotide of the invention may be engineered for expression in a replication defective retroviral vector, as discussed above. The retroviral expression construct then may be isolated and introduced into a packaging cell is transduced with a retroviral plasmid vector containing RNA encoding a polypeptide of the present invention such that the packaging cell now produces infectious viral particles containing the gene of interest. These producer cells may be administered to a patient for engineering cells *in vivo* and expression of the polypeptide *in vivo*. These and other methods for administering a polypeptide of the present invention by such method should be apparent to those skilled in the art from the teachings of the present invention.

Retroviruses from which the retroviral plasmid vectors herein above mentioned may be derived include, but are not limited to, Moloney Murine Leukemia Virus, spleen necrosis virus, retroviruses such as Rous Sarcoma Virus, Harvey Sarcoma Virus, avian leukosis virus, gibbon ape leukemia virus, human immunodeficiency virus, adenovirus, Myeloproliferative Sarcoma Virus, and mammary tumor virus. In one embodiment, the retroviral plasmid vector is derived from Moloney Murine Leukemia Virus.

Such vectors well include one or more promoters for expressing the polypeptide. Suitable promoters which may be employed include, but are not limited to, the retroviral LTR; the SV40 promoter; and the human cytomegalovirus (CMV) promoter described in Miller et al., Biotechniques 7: 980-990 (1989), or any other promoter (e.g., cellular promoters such as eukaryotic cellular promoters including, but not limited to, the histone, RNA polymerase III, and ß-actin promoters). Other viral promoters which may be employed include, but are not limited to, adenovirus promoters, thymidine kinase (TK) promoters, and B19 parvovirus promoters. The selection of a suitable promoter will be apparent to those skilled in the art from the teachings contained herein.

The nucleic acid sequence encoding the polypeptide of the present invention will be placed under the control of a suitable promoter. Suitable promoters which may be employed include, but are not limited to, adenoviral promoters, such as the adenoviral major late promoter; or heterologous promoters, such as the cytomegalovirus (CMV) promoter; the respiratory syncytial virus (RSV) promoter; inducible promoters, such as the MMT promoter, the metallothionein promoter; heat shock promoters; the albumin promoter; the ApoAI promoter; human globin promoters; viral thymidine kinase promoters, such as the Herpes Simplex thymidine kinase promoter; retroviral LTRs (including the modified retroviral LTRs herein above described); the ß-actin promoter; and human growth hormone promoters. The promoter also may be the native promoter which controls the gene encoding the polypeptide.

The retroviral plasmid vector is employed to transduce packaging cell lines to form producer cell lines. Examples of packaging cells which may be transfected include, but are not limited to, the PE501, PA317, Y-2, Y-AM, PA12, T19-14X, VT-19-17-H2, YCRE, YCRIP, GP+E-86, GP+envAm12, and DAN cell lines as described in Miller, A., Human Gene Therapy 1: 5-14 (1990). The vector may be transduced into the packaging cells through any means known in the art. Such means include, but are not limited to, electroporation, the use of liposomes, and CaPO4 precipitation. In one alternative, the retroviral plasmid vector may be encapsulated into a liposome, or coupled to a lipid, and then administered to a host.

The producer cell line will generate infectious retroviral vector particles, which include the nucleic acid sequence(s) encoding the polypeptides. Such retroviral vector particles then may be employed to transduce eukaryotic cells, either in vitro or *in vivo*. The transduced eukaryotic cells will express the nucleic acid sequence(s) encoding the polypeptide. Eukaryotic cells which may be transduced include, but are not limited to, embryonic stem cells, embryonic carcinoma cells, as well as hematopoietic stem cells, hepatocytes, fibroblasts, myoblasts, keratinocytes, endothelial cells, and bronchial epithelial cells.

EXAMPLES

The present invention is further described by the following examples. The examples are provided solely to illustrate the invention by reference to specific embodiments. These exemplification's, while illustrating certain specific aspects of the invention, do not portray the limitations or circumscribe the scope of the disclosed invention.

Certain terms used herein are explained in the foregoing glossary.

All examples were carried out using standard techniques, which are well known and routine to those of skill in the art, except where otherwise described in detail. Routine molecular biology techniques of the following examples can be carried out as described in standard laboratory manuals, such as Sambrook et al., MOLECULAR CLONING: A LABORATORY MANUAL, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989), herein referred to as "Sambrook."

All parts or amounts set out in the following examples are by weight, unless otherwise specified.

Unless otherwise stated size separation of fragments in the examples below was carried out using standard techniques of agarose and polyacrylamide gel electrophoresis ("PAGE") in Sambrook and numerous other references such as, for instance, by Goeddel et al., Nucleic Acids Res. 8: 4057 (1980).

Unless described otherwise, ligations were accomplished using standard buffers, incubation temperatures and times, approximately equimolar amounts of the DNA fragments to be ligated and approximately 10 units of T4 DNA ligase ("ligase") per 0.5 µg of DNA..

SEQUENCE LISTING

(1) GENERAL INFORMATION

(i) APPLICANTS: SmithKline Beecham Corporation and Human Genome Sciences, Inc.

(ii) TITLE OF THE INVENTION: HR-1 RECEPTOR

(iii) NUMBER OF SEQUENCES: 2

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: SmithKline Beecham Corporation
(B) STREET: 709 Swedeland Road
(C) CITY: King of Prussia
(D) STATE: PA
(E) COUNTRY: USA
(F) ZIP: 19406-2799

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: DOS
(D) SOFTWARE: FastSEQ Version 1.5

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Han, William T
(B) REGISTRATION NUMBER: 34,344
(C) REFERENCE/DOCKET NUMBER: ATG50007

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 610-270-5219
(B) TELEFAX: 610-270-5090
(C) TELEX:

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1288 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GGCAATATCA AGGTTTTAAA TCTCGGAGAA ATGGCTTTCG TTTGCTTGGC TATCGGATGC 60
TTATATACCT TTCTGATAAG CACAACATTT GGCTGTACTT CATCTTCAGA CACCGAGATA 120
AAAGTTAACC CTCCTCAGGA TTTTGAGATA GTGGATCCCG GATACTTAGG TTATCTCTAT 180
TTGCAATGGC AACCCCCACT GTCTCTGGAT CATTTTAAGG AATGCACAGT GGAATATGAA 240
CTAAAATACC GAAACATTGG TAGTGAAACA TGGAAGACCA TCATTACTAA GAATCTACAT 300
TACAAAGATG GGTTTGATCT TAACAAGGGC ATTGAAGCGA AGATACACAC GCTTTTACCA 360
TGGCAATGCA CAAATGGATC AGAAGTTCAA AGTTCCTGGG CAGAAACTAC TTATTGGATA 420
TCACCACAAG GAATTCCAGA AACTAAAGTT CAGGATATGG ATTGCGTATA TTACAATTGG 480
CAATATTTAC TCTGTTCTTG GAAACCTGGC ATAGGTGTAC TTCTTGATAC CAATTACAAC 540
TTGTTTTACT GGTATGAGGG CTTGGATCAT GCATTACAGT GTGTTGATTA CATCAAGGCT 600

GATGGACAAA ATATAGGATG CAGATTTCCC TATTTGGAGG CATCAGACTA TAAAGATTTC 660
TATATTTGTG TTAATGGATC ATCAGAGAAC AAGCCTATCA GATCCAGTTA TTTCACTTTT 720
CAGCTTCAAA ATATAGTTAA ACCTTTGCCG CCAGTCTATC TTACTTTTAC TCGGGAGAGT 780
TCATGTGAAA TTAAGCTGAA ATGGAGCATA CCTTTGGGAC CTATTCCAGC AAGGTGTTTT 840
GATTATGAAA TTGAGATCAG AGAAGATGAT ACTACCTTGG TGACTGCTAC AGTTGAAAAT 900
GAAACATACA CCTTGAAAAC AACAAATGAA ACCCGACAAT TATGCTTTGT AGTAAGAAGC 960
AAAGTGAATA TTTATTGCTC AGATGACGGA ATTTGGAGTG AGTGGAGTGA TAAACAATGC 1020
TGGGAAGGTG AAGACCTATC GAAGAAAACT TTGCTACGTT TCTGGCTACC ATTTGGTTTC 1080
ATCTTAATAT TAGTTATATT TGTAACCGGT CTGCTTTTGC GTAAGCCAAA CACCTACCCA 1140
AAAATGATTC CAGAATTTTT CTGTGATACA TGAAGACTTT CCATATCAAG AGACATGGTA 1200
TTGACTCAAC AGTTTCCAGT CATGGCCAAA TGTTCAATAT GAGTCTCAAT AAACTGAATT 1260
TTTCTTGCGA AAAAAAAAAA AAAAAAAA 1288

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 380 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE: N-terminal
(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Ala Phe Val Cys Leu Ala Ile Gly Cys Leu Tyr Thr Phe Leu Ile
1 5 10 15
Ser Thr Thr Phe Gly Cys Thr Ser Ser Ser Asp Thr Glu Ile Lys Val
20 25 30
Asn Pro Pro Gln Asp Phe Glu Ile Val Asp Pro Gly Tyr Leu Gly Tyr
35 40 45
Leu Tyr Leu Gln Trp Gln Pro Pro Leu Ser Leu Asp His Phe Lys Glu
50 55 60

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Cys Thr Val Glu Tyr Glu Leu Lys Tyr Arg Asn Ile Gly Ser Glu Thr
65                  70                  75                  80
Trp Lys Thr Ile Ile Thr Lys Asn Leu His Tyr Lys Asp Gly Phe Asp
                85                  90                  95
Leu Asn Lys Gly Ile Glu Ala Lys Ile His Thr Leu Leu Pro Trp Gln
            100                 105                 110
Cys Thr Asn Gly Ser Glu Val Gln Ser Ser Trp Ala Glu Thr Thr Tyr
        115                 120                 125
Trp Ile Ser Pro Gln Gly Ile Pro Glu Thr Lys Val Gln Asp Met Asp
    130                 135                 140
Cys Val Tyr Tyr Asn Trp Gln Tyr Leu Leu Cys Ser Trp Lys Pro Gly
145                 150                 155                 160
Ile Gly Val Leu Leu Asp Thr Asn Tyr Asn Leu Phe Tyr Trp Tyr Glu
                165                 170                 175
Gly Leu Asp His Ala Leu Gln Cys Val Asp Tyr Ile Lys Ala Asp Gly
            180                 185                 190
Gln Asn Ile Gly Cys Arg Phe Pro Tyr Leu Glu Ala Ser Asp Tyr Lys
        195                 200                 205
Asp Phe Tyr Ile Cys Val Asn Gly Ser Ser Glu Asn Lys Pro Ile Arg
    210                 215                 220
Ser Ser Tyr Phe Thr Phe Gln Leu Gln Asn Ile Val Lys Pro Leu Pro
225                 230                 235                 240
Pro Val Tyr Leu Thr Phe Thr Arg Glu Ser Ser Cys Glu Ile Lys Leu
                245                 250                 255
Lys Trp Ser Ile Pro Leu Gly Pro Ile Pro Ala Arg Cys Phe Asp Tyr
            260                 265                 270
Glu Ile Glu Ile Arg Glu Asp Asp Thr Thr Leu Val Thr Ala Thr Val
        275                 280                 285
Glu Asn Glu Thr Tyr Thr Leu Lys Thr Thr Asn Glu Thr Arg Gln Leu
    290                 295                 300
Cys Phe Val Val Arg Ser Lys Val Asn Ile Tyr Cys Ser Asp Asp Gly
305                 310                 315                 320
Ile Trp Ser Glu Trp Ser Asp Lys Gln Cys Trp Glu Gly Glu Asp Leu
                325                 330                 335
Ser Lys Lys Thr Leu Leu Arg Phe Trp Leu Pro Phe Gly Phe Ile Leu
            340                 345                 350
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Ile Leu Val Ile Phe Val Thr Gly Leu Leu Leu Arg Lys Pro Asn Thr
        355                 360                 365
Tyr Pro Lys Met Ile Pro Glu Phe Phe Cys Asp Thr
    370                 375                 380
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What is claimed is:

1. An isolated polynucleotide comprising a member selected from the group consisting of:

(a) a polynucleotide having at least a 70% identity to a polynucleotide encoding a polypeptide comprising amino acids 1 to 380 of SEQ ID NO:2;

(b) a polynucleotide having at least a 70% identity to a polynucleotide encoding a polypetide comprising amino acids 22 to 380 of SEQ ID NO: 2;

(c) a polynucleotide which is complementary to the polynucleotide of (a) or (b); and

(c) a polynucleotide comprising at least 15 bases of the polynucleotide of (a), (b) or (c).

2. The polynucleotide of Claim 1 wherein the polynucleotide is DNA.

3. The polynucleotide of Claim 1 wherein the polynucleotide is RNA.

4. The polynucleotide of Claim 2 comprising nucleotides 1 to 1288 set forth in SEQ ID NO:1.

5. The polynucleotide of Claim 2 comprising nucleotides 31 to 1170 set forth in SEQ ID NO:1.

6. The polynucleotide of Claim 2 which encodes a polypeptide comprising amino acids 1 to 380 of SEQ ID NO:2.

7. The polynucleotide of Claim 2 which encodes a polypeptide comprising amino acids 22 to 380 of SEQ ID NO:2.

8. An isolated polynucleotide comprising a member selected from the group consisting of:

(a) a polynucleotide having at least a 70% identity to a polynucleotide encoding the same mature polypeptide expressed by the human cDNA contained in ATCC Deposit No. 98069;

(b) a polynucleotide complementary to the polynucleotide of (a); and

(c) a polynucleotide comprising at least 15 bases of the polynucleotide of (a) or (b).

9. A vector comprising the DNA of Claim 2.

10. A host cell comprising the vector of Claim 9.

11. A process for producing a polypeptide comprising: expressing from the host cell of Claim 10 a polypeptide encoded by said DNA.

12. A process for producing a cell which expresses a polypeptide comprising transforming or transfecting the cell with the vector of Claim 9 such that the cell expresses the polypeptide encoded by the human cDNA contained in the vector.

13. A polypeptide comprising an amino acid sequence which is at least 70% identical to amino acid 1 to 380 of SEQ ID NO:2.

14. A polypeptide comprising an amino acid sequence which is at least 70% identical to amino acid 22 to 380 of SEQ ID NO:2.

15. A polypeptide comprising an animo acid sequence 1 to 380 as set forth in SEQ ID NO:2.

16. A polypeptide comprising an animo acid sequence 22 to 380 as set forth in SEQ ID NO:2.

17. An agonist to the polypeptide of claim 13, 14, 15 or 16.

18. An antibody against the polypeptide of claim 13, 14, 15 or 16.

19. An antagonist which inhibits the activity of the polypeptide of claim 13, 14, 15 or 16.

20. A method for the treatment of a patient having need of HR-1 RECEPTOR comprising: administering to the patient a therapeutically effective amount of the polypeptide of claim 13, 14, 15 or 16.

21. The method of Claim 20 wherein said therapeutically effective amount of the polypeptide is administered by providing to the patient DNA encoding said polypeptide and expressing said polypeptide *in vivo*.

22. A method for the treatment of a patient having need to inhibit HR-1 RECEPTOR polypeptide comprising: administering to the patient a therapeutically effective amount of the antagonist of Claim 19.

23. A process for diagnosing a disease or a susceptibility to a disease related to expression of the polypeptide of claim 13, 14, 15 or 16 comprising:

determining a mutation in the nucleic acid sequence encoding said polypeptide.

24. A diagnostic process comprising:

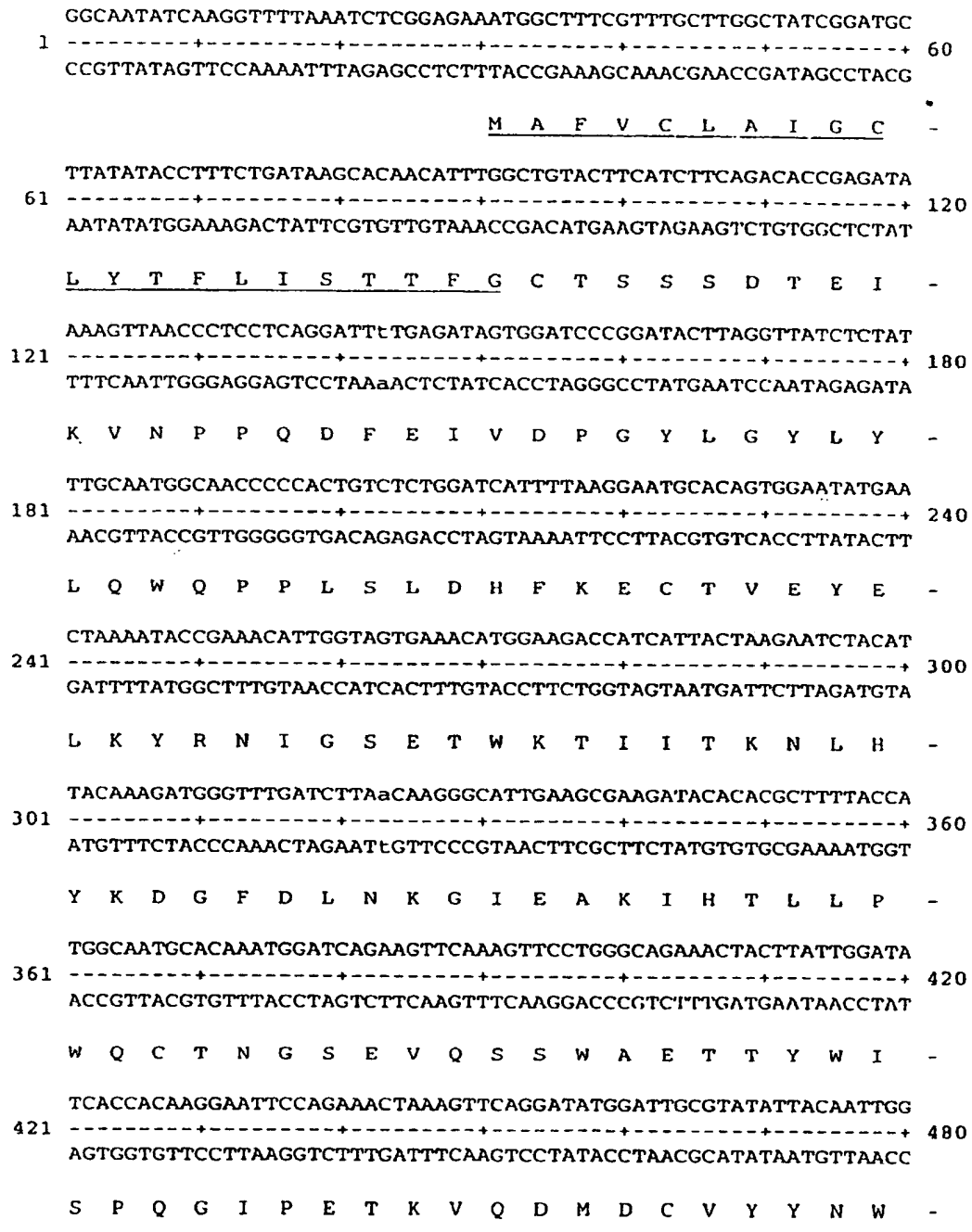
analyzing for the presence of the polypeptide of claim 13, 14, 15 or 16 in a sample derived from a host.

25. A method for identifying compounds which bind to and activate or inhibit a receptor for the polypeptide of claim 13, 14, 15 or 16 comprising:

contacting a cell expressing on the surface thereof a receptor for the polypeptide, said receptor being associated with a second component capable of providing a detectable signal in response to the binding of a compound to said receptor, with a compound to be screened under conditions to permit binding to the receptor; and

determining whether the compound binds to and activates or inhibits the receptor by detecting the presence or absence of a signal generated from the interaction of the compound with the receptor.

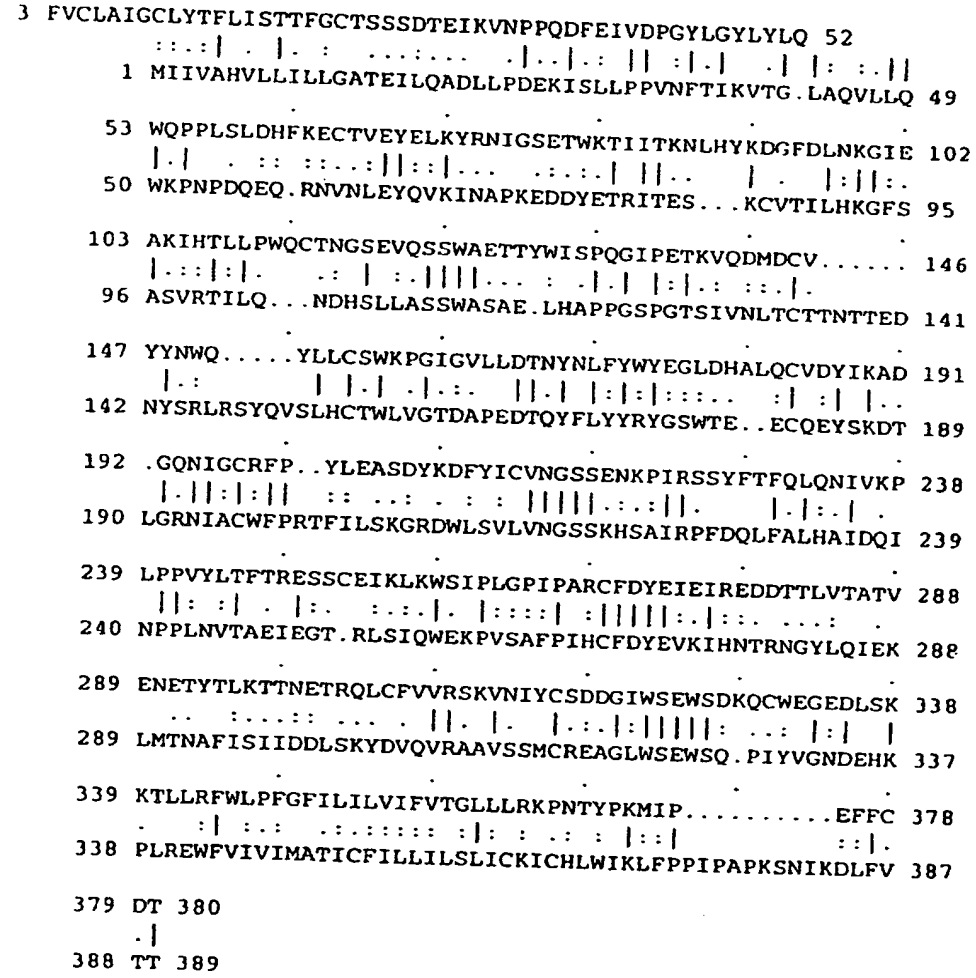
FIGURE 1.



```
    CAATATTTACTCTGTTCTTGGAAACCTGGCATAGGTGTACTTCTTGATACCAATTACAAC
481 ---------+---------+---------+---------+---------+---------+ 540
    GTTATAAATGAGACAAGAACCTTTGGACCGTATCCACATGAAGAACTATGGTTAATGTTG

    Q  Y  L  L  C  S  W  K  P  G  I  G  V  L  L  D  T  N  Y  N  -

    TTGTTTTACTGGTATGAGGGCTTGGATCATGCATTACAGTGTGTTGATTACATCAAGGCT
541 ---------+---------+---------+---------+---------+---------+ 600
    AACAAAATGACCATACTCCCGAACCTAGTACGTAATGTCACACAACTAATGTAGTTCCGA

    L  F  Y  W  Y  E  G  L  D  H  A  L  Q  C  V  D  Y  I  K  A  -

    GATGGACAAAATATAGGATGCAGATTTCCCTATTTGGAGGCATCAGACTATAAAGATTTC
601 ---------+---------+---------+---------+---------+---------+ 660
    CTACCTGTTTTATATCCTACGTCTAAAGGGATAAACCTCCGTAGTCTGATATTTCTAAAG

    D  G  Q  N  I  G  C  R  F  P  Y  L  E  A  S  D  Y  K  D  F  -

    TATATTTGTGTTAATGGATCATCAGAGAACAAGCCTATCAGATCCAGTTATTTCACTTTT
661 ---------+---------+---------+---------+---------+---------+ 720
    ATATAAACACAATTACCTAGTAGTCTCTTGTTCGGATAGTCTAGGTCAATAAAGTGAAAA

    Y  I  C  V  N  G  S  S  E  N  K  P  I  R  S  S  Y  F  T  F  -

    CAGCTTCAAAATATAGTTAAACCTTTGCCGCCAGTCTATCTTACTTTTACTCGGGAGAGT
721 ---------+---------+---------+---------+---------+---------+ 780
    GTCGAAGTTTTATATCAATTTGGAAACGGCGGTCAGATAGAATGAAAATGAGCCCTCTCA

    Q  L  Q  N  I  V  K  P  L  P  P  V  Y  L  T  F  T  R  E  S  -

    TCATGTGAAATTAAGCTGAAATGGAGCATACCTTTGGGACCTATTCCAGCAAGGTGTTTT
781 ---------+---------+---------+---------+---------+---------+ 840
    AGTACACTTTAATTCGACTTTACCTCGTATGGAAACCCTGGATAAGGTCGTTCCACAAAA

    S  C  E  I  K  L  K  W  S  I  P  L  G  P  I  P  A  R  C  F  -

    GATTATGAAATTGAGATCAGAGAAGATGATACTACCTTGGTGACTGCTACAGTTGAAAAT
841 ---------+---------+---------+---------+---------+---------+ 900
    CTAATACTTTAACTCTAGTCTCTTCTACTATGATGGAACCACTGACGATGTCAACTTTTA

    D  Y  E  I  E  I  R  E  D  D  T  T  L  V  T  A  T  V  E  N  -

    GaAACATACACCtTGAAAACAACaAATGAAACCCGACAATTATGCTTTGTAGTAAGAAGC
901 ---------+---------+---------+---------+---------+---------+ 960
    CtTTGTATGTGGaACTTTTGTTGtTTACTTTGGGCTGTTAATACGAAACATCATTCTTCG

    E  T  Y  T  L  K  T  T  N  E  T  R  Q  L  C  F  V  V  R  S  -

    AAAGTGAATATTTATTGCTCAGATGACGGAATTTGGAGTGAGTGGAGTGATAAACAATGC
961 ---------+---------+---------+---------+---------+---------+ 1020
    TTTCACTTATAAATAACGAGTCTACTGCCTTAAACCTCACTCACCTCACTATTTGTTACG

    K  V  N  I  Y  C  S  D  D  G  I  W  S  E  W  S  D  K  Q  C  -
```

```
     TGGGAAGGTGAAGACCTATCGAAGAAAACTTTGCTACGTTTCTGGCTACCATTTGGTTTC
1021 ---------+---------+---------+---------+---------+---------+ 1080
     ACCCTTCCACTTCTGGATAGCTTCTTTTGAAACGATGCAAAGACCGATGGTAAACCAAAG

     W  E  G  E  D  L  S  K  K  T  L  L  R  F  W  L  P  F  G  F  -

     ATCTTAATATTAGTTATATTTGTAACCGGTCTGCTTTTGCGTAAGCCAAACACCTACCCA
1081 ---------+---------+---------+---------+---------+---------+ 1140
     TAGAATTATAATCAATATAAACATTGGCCAGACGAAAACGCATTCGGTTTGTGGATGGGT

     I  L  I  L  V  I  F  V  T  G  L  L  L  R  K  P  N  T  Y  P  -

     AAAATGATTCCAGAATTTTTCTGTGATACATGAAGACTTTCCATATCAAGAGACATGGTA
1141 ---------+---------+---------+---------+---------+---------+ 1200
     TTTTACTAAGGTCTTAAAAAGACACTATGTACTTCTGAAAGGTATAGTTCTCTGTACCAT

     K  M  I  P  E  F  F  C  D  T  *                              -

     TTGACTCAACAGTTTCCAGTCATGGCCAAATGTTCAATATGAGTCTCAATAAACTGAATT
1201 ---------+---------+---------+---------+---------+---------+ 1260
     AACTGAGTTGTCAAAGGTCAGTACCGGTTTACAAGTTATACTCAGAGTTATTTGACTTAA

                                                                   -

     TTTCTTGCGAAAAAAAAAAAAAAAAAA
1261 ---------+---------+-------- 1288
     AAAGAACGCTTTTTTTTTTTTTTTTTT
```

Figure 2


3 FVCLAIGCLYTFLISTTFGCTSSSDTEIKVNPPQDFEIVDPGYLGYLYLQ 52
1 MIIVAHVLLILLGATEILQADLLPDEKISLLPPVNFTIKVTG.LAQVLLQ 49
53 WQPPLSLDHFKECTVEYELKYRNIGSETWKTIITKNLHYKDGFDLNKGIE 102
50 WKPNPDQEQ.RNVNLEYQVKINAPKEDDYETRITES...KCVTILHKGFS 95
103 AKIHTLLPWQCTNGSEVQSSWAETTYWISPQGIPETKVQDMDCV..... 146
96 ASVRTILQ...NDHSLLASSWASAE.LHAPPGSPGTSIVNLTCTTNTTED 141
147 YYNWQ.....YLLCSWKPGIGVLLDTNYNLFYWYEGLDHALQCVDYIKAD 191
142 NYSRLRSYQVSLHCTWLVGTDAPEDTQYFLYYRYGSWTE..ECQEYSKDT 189
192 .GQNIGCRFP..YLEASDYKDFYICVNGSSENKPIRSSYFTFQLQNIVKP 238
190 LGRNIACWFPRTFILSKGRDWLSVLVNGSSKHSAIRPFDQLFALHAIDQI 239
239 LPPVYLTFTRESSCEIKLKWSIPLGPIPARCFDYEIEIREDDTTLVTATV 288
240 NPPLNVTAEIEGT.RLSIQWEKPVSAFPIHCFDYEVKIHNTRNGYLQIEK 288
289 ENETYTLKTTNETRQLCFVVRSKVNIYCSDDGIWSEWSDKQCWEGEDLSK 338
289 LMTNAFISIIDDLSKYDVQVRAAVSSMCREAGLWSEWSQ.PIYVGNDEHK 337
339 KTLLRFWLPFGFILILVIFVTGLLLRKPNTYPKMIP.....EFFC 378
338 PLREWFVIVIMATICFILLILSLICKICHLWIKLFPPIPAPKSNIKDLFV 387
379 DT 380
388 TT 389

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/10262

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C12N 15/12; C07K 14/705, 14/715; A61K 38/17
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/350; 435/69.1, 71.1, 71.2, 172.3, 240.1, 252.3, 320.1; 536/23.1, 23.5, 24.3; 514/2, 12

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, CAS ONLINE, MEDLINE, BIOSIS
search terms: cytokine/peptide hormone receptor, production or isolation, treatment or administration or therapy.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SCIENCE, Volume 257, issued 17 July 1992, Takeshita et al, "Cloning of the gamma Chain of the Human IL-2 Receptor", pages 379-382, see entire document.	1-16, 20
A	GENE, Volume 130, issued 1993, Kobayashi et al, "Cloning and Sequencing of the cDNA encoding a Mouse IL-2 Receptor gamma", pages 303-304, see entire document.	1-16, 20
A	JOURNAL OF EXPERIMENTAL MEDICINE, Volume 175, issued February 1992, Murata et al, "Molecular Cloning and Expression of the Human Interleukin 5 Receptor", pages 341-351, see pages 343-345.	1-16, 20

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 12 AUGUST 1996	Date of mailing of the international search report 03 OCT 1996
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer PREMA MERTZ Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/10262

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GENE, Volume 134, issued 1993, Moore et al, "Cloning and Sequencing of the cDNA Encoding the Murine Mammary Gland Long-Form Prolactin Receptor", pages 263-265, see entire document.	1-16, 20
A	THE JOURNAL OF IMMUNOLOGY, Volume 148, Number 12, issued 15 June 1992, Saito et al, "Molecular Cloning of a Murine IL-6 ReceptoAssociated Signal Transducer, gp130, and its Regulated Expression in vivo", pages 4066-4071, see pages 4067-4069.	1-16, 20

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/10262

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-16, 20

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/10262

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

530/350; 435/69.1, 71.1, 71.2, 172.3, 240.1, 252.3, 320.1; 536/23.1, 23.5, 24.3; 514/2, 12

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claims 1-16 and 20, drawn to a polynucleotide encoding human HR-1 receptor, vector, host cell, a process for producing the polypeptide, human HR-1 receptor polypeptide and a method of treatment by administering human HR-1 receptor polypeptide.
Group II, claim 17, drawn to an agonist to the human HR-1 receptor polypeptide.
Group III, claim 18, drawn to an antibody to the human HR-1 receptor polypeptide.
Group IV, claims 19 and 22, drawn to an antagonist which inhibits the activity of the human HR-1 receptor polypeptide and a method of treatment by administering the antagonist.
Group V, claim 21, drawn to a method of treatment by administering DNA encoding human HR-1 receptor polypeptide.
Group VI, claim 23, drawn to a process of diagnosing a disease related to expression of the human HR-1 receptor polypeptide by determining a mutation in the nucleic acid sequence encoding the polypeptide.
Group VII, claim 24, drawn to a diagnostic process comprising analyzing for the presence of the human HR-1 receptor polypeptide derived from a host.
Group VIII, claim 25, drawn to a method for identifying compounds which bind to and activate or inhibit a receptor for the HR-1receptor polypeptide.
The inventions listed as Groups I-VIII do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:
The products/processes of Groups I-IV do not share the same or a corresponding technical feature in that the polynucleotides encoding human HR-1 receptor polypeptide, vector, host cell, a process for producing the polypeptide, human HR-1 receptor polypeptide, and a method of treatment by administering human HR-1 receptor polypeptide of Group I, the agonist of Group II, the antibody of Group III, the antagonist and a method of treatment by administering the antagonist of Group IV do not require each other for their practice and have separate functions all of which constitute the special technical features which define the contribution of each invention. Since these special technical features are not shared by each product and since the common features do not establish an advance over the prior art, the inventions of Groups I-IV do not form a single inventive concept within the meaning of Rule 13.2.
The processes of Groups V-VIII do not share the same or a corresponding technical feature in that a method of treatment by administering DNA encoding human HR-1 receptor polypeptide of Group V, a method of diagnosing a disease related to expression of the human HR-1 receptor polypeptide by determining a mutation in the nucleic acid sequence encoding the polypeptide of Group VI, a diagnostic process comprising analyzing forthepresenceofthehumanHR-1 receptor polypeptide from a host of Group VII and a method for identifying compounds which bind to and activate or inhibit a receptor for human HR-1 receptor polypeptide, do not require each other for their practice, have separate uses and different method steps all of which constitute the special technical features which define the contribution of each invention. Since these special technical features are not shared by each process and since the common features do not establish an advance over the prior art, the inventions of Groups V-VIII do not share a technical relationship and do not form a single inventive concept within the meaning of Rule 13.2.
The inventions of Groups II-IV do not share the same or a corresponding technical feature with the inventions of Groups V-VIII because the inventions of Groups II-IV are not used or produced by the inventions of Groups V-VIII.
The invention of Group I is separate and distinct from the inventions of Groups V or VI because the DNA of Group I may be used in other methods other than the method of treatment with DNA or a process of diagnosing a disease. The DNA of Group I can be used in the production of the protein of interest.